H.C.

THE TOHOKU JOURNAL OF EXPERIMENTAL MEDICINE

VOL. 50, Nos. 3-4, June 30, 1949

OSAKA CITY UNIVERSITY
NOV - 8 1960
L.

EDITORIAL BOARD

H. MASAMUNE K. MOTOKAWA Y. SATAKE

WITH COLLABORATION OF

T. EBINA, T. FUJITA, Y. HAYASI, S. IINO,
T. ISHIKAWA, M. ITO, T. KATO, S.-T. KATSURA,
T. KOGA, S. KONDO, Y. KOYANAGI, T. KUMAGAI,
M. KUROYA, K. MARUI, K. MATSUDA, T. MURAKAMI,
Y. NAKAZAWA, S. NASU, S. OSATO, T. SANO, A. SATO,
S. SATO, H. SETO, Y. SHINODA, SH. SUGIMURA,
Y. TUIKI, M. WADA, S. YAGI, M. YAMASAKI,
T. YOSHIDA.

PUBLISHED BY
THE TOHOKU UNIVERSITY.
SENDAI, JAPAN.

SOLD BY
MARUZEN COMPANY, LTD., TOKYO.

The Tohoku Journal of Experimental Medicine is an official organ of the Tohoku University for the publication of records of original research in all branches of experimental medicine, but accepts also papers from sources other than the University, domestic as well as overseas, under certain limitations, given below.

Three to four volumes, each containing four numbers, are issued a year. Each volume will consist of approximately 400 pages.

The articles are written in English, French or German.

The subscription price is $5.00, foreign $5.50, per volume, postpaid, and should be sent to the Maruzen Co., Ltd., Tokyo.

Papers and correspondence regarding exchange of publications, etc. should be addressed to the Medical Library, Tohoku University, Kita-yoban-tyo, Sendai.

Notice to Contributors.

Papers will not be accepted for simultaneous publication or those which have been previously published in the periodicals written in English, French, German or Italian. All articles from Laboratories must be approved by the director of the originating laboratory. Contributions should be ordinarily limited in length to 8 printed pages. Every paper must be accompanied by a brief summary.

The manuscripts should be typewritten in double spacing, and carefully revised before submission to the Editor. The original, not carbon, copy should be sent. Bibliographies should give the name of the author, the name of journal or book (with edition & place of publishing), the year, the volume and the page.

Figures should be ready for photographic reproduction, diagrams should be drawn in Indian ink and plain white or fine blue-lined paper only should be employed; letters, numbers etc. should be written in pencil. The reduction desired should be clearly indicated on the margin of the drawing. The Journal cannot accept illustrations which when reproduced for printing exceed the size of a Journal page. Authors should indicate on the manuscript the approximate position of the text figures and tables.

All the drawings and tables should be marked with the author's name. They should be packed flate, carefully protected from injury, and insured.

Proofs will not be submitted to authors overseas unless they express a desire to see them, or unless the editors think it necessary. Overseas authors are therefore particularly reminded of the importance of preparing their manuscripts in such a way that error need not arise.

50 reprints of articles will be furnished to authors gratis.

The Tohoku Journal of Experimental Medicine, Vol. 50, Nos. 3 & 4, 1949.

Experimentelle Untersuchungen über das Schicksal der Tuberkelbazillen im Blutstrom, insbesondere über ihre Ausscheidungswege.

Mitteilung II: Über die Verbreitung der in die Ohrvene von Kaninchen injizierten Tuberkelbazillen in der Leber, insbesondere über ihr Auftreten im Gallengang der Kaninchen.

Von

Takesi Abe.

(阿 部 武)

(Aus der Medizinischen Klinik und dem Kosankinbyo Kenkyusio der Kaiserlichen Tohoku-Universität zu Sendai, Leiter: Prof. Dr. T. Kumagai und Prof. Dr. T. Ebina.)

(Received for publication, April 4, 1945)

1. Einleitung.

Calmette und Guérin[1] haben nach Ablauf von 3 und 4 Tagen nach der Injektion von Tuberkelbazillen in die Ohrvene von Kaninchen die Ausscheidung der Bazillen in der Galle nachgewiesen und sie auf die physiologischen Ausscheidung der Leber zurückgeführt. Rabinowitsch[2] und andere behaupteten dagegen, dass die Tuberkelbazillen infolge des Durchbruchs des tuberkulösen Herdes im Gallengang in der Galle auftreten. Diese Frage scheint also heute noch nicht endgültig gelöst zu sein. Die Autoren haben aber fast alle nur auf den Nachweis von Tuberkelbazillen in der Galle allein untersucht und einfach geglaubt, dass sie aus der Leber ausgeschieden werden. Es ist jedoch bisher noch kein Versuch angestellt, um festzustellen, ob die Tuberkelbazillen wirklich in Lebergewebe ausgeschieden werden. Da über die Herkunft der Bakterien in der Galle neben der Ausscheidung aus der Leber die Auffassung stark gilt, dass die Bakterien direkt aus der Schleimhaut der Gallenblase ausgeschieden werden, so braucht man die Tuberkelbazillen direkt aus dem Gallengang in der Leber nachzuweisen, wenn man anders beweisen will, dass sie wirklich aus der Leber ausgeschieden werden.

In vorliegender Arbeit wollen wir bei den Kaninchen mit Bazillämie die Verbreitung der Tuberkelbazillen in der Leber und ihr Schicksal untersuchen und vor allem feststellen, ob die Bazillen im interlobulären Gallengang ausgeschieden werden.

2. Versuchsmethode und -material.

Als Versuchstiere wurden die 37 gesunden Kaninchen mit negativer Tuberkulinreaktion, die in der 1. Mitteilung gebraucht wurden, benutzt. Sie bekamen 1 oder 10 mg Tuberkelbazillen durch die Einspritzung in die Ohrvene und wurden in bestimmten Zeiträumen durch die Luftembolie getötet. Dann fixierte man ein Stück aus Lebergewebe und färbte es nach der Haematoxylin-Eosin-Färbung, der van Giesonschen und der von Katura modifizierten Tuberkelbazillenfärbung von Wada,[3] um sie dann zu mikroskopieren. Bei der Sektion von den Fällen, denen 10 mg Bazillen eingespritzt wurde, entnahm man die Galle mittelst einer aseptischen Spritze durch die Punktion aus der Gallenblase. Nach Zusatz mit 10 ccm von 2% NaOH und 1 ccm 2.5% $ZnSO_4$ wurde sie zentrifugiert. Der Niederschlag wurde mit 2–3 ccm 4% H_2SO_4 behandelt und auf dem Oka-Katakura schen Nährboden gezüchtet.

3. Versuchsresultate.

1) Hyperämie des Lebergewebes. Die Hyperämie des Leber-

TABELLE I.

Leberbefund der Kaninchen mit der Bazillämie.

Kaninchen Nr.			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Inokulierte Dosis			1 mg																	
Infektionsdauer			3 st	〃	6	〃	24	〃	2 T	〃	3	〃	7	〃	10	14	14	21	28	35
Körpergewicht			kg 1.5	1.5	1.6	1.7	1.7	1.7	1.5	1.7	1.9	2.7	2.1	2.7	2.1	2.2	2.8	2.5	2.2	2.3
Tuberc. -Reaktion			−	−	−	−	−	−	−	−	−	−	−	--	+	+	+	+	+	+
Makrospopische Tuberkel			−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Mikroskopische Befunde	Hyperämie		+	+	±	+	+	+	±	+	±	±	−	±	±	−	−	−	−	−
	Degeneration der Leberzellen		+	±	−	+	+	±	+	−	−	−	+	−	−	±	−	−	−	−
	Tuberkelbildung	Polymorph. Leukozyt.	−	−	−	+	−	+	+	±	±	−	−	−	−	−	−	−	−	−
		Sternzellen, Hystiozyt.	−	−	±	±	+	−	±	+	±	−	±	−	++	−	+	−	−	−
		Epitheloidzellen	−	−	−	−	+	−	−	+	±	+	−	+	+	−	−	+	+	+
		Lymphozyten	−	−	−	−	+	−	−	+	±	+	−	+	+	−	−	+	+	+
		Riesenzellen	−	−	−	−	−	−	−	−	−	+	−	+	+	−	−	+	+	−
		Nekrose	−	−	−	−	−	−	−	+	±	±	−	±	−	−	−	−	−	−
		kollagene Fasern	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
		Kalkablagerung	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

TABELLE I.

(Fortgesetzt)

Kaninchen Nr.		19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37
Inokulierte Dosis		10 mg																		
Infektionsdauer		1 st	3	6	24	2 T	3	7	8	10	11	13	14	16	21	28	34	41	41	44
Körpergewicht		1.5	1.7	1.9	1.4	2.0	1.9	2.1	1.4	2.0	1.5	2.3	1.4	2.0	2.1	1.5	1.4	2.5	2.2	1.5
Tuberc. -Reaktion		–	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+
Makrospopische Tuberkel		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Mikroskopische Befunde	Hyperämie	–	–	–	–	–	±	–	+	–	+	±	–	–	–	–	–	–	–	–
	Degeneration der Leberzellen	±	+	±	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Tuberkelbildung: Polymorph. Leukozyt.	–	+	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–
	Tuberkelbildung: Sternzellen, Hystiozyt.	–	+	–	±	–	±	–	–	–	–	–	–	–	–	–	–	–	–	–
	Tuberkelbildung: Epitheloidzellen	–	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+
	Tuberkelbildung: Lymphozyten	–	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+
	Tuberkelbildung: Riesenzellen	–	–	–	–	–	–	–	+	+	+	–	–	+	+	+	+	+	+	+
	Tuberkelbildung: Nekrose	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
	Tuberkelbildung: kollagene Fasern	–	–	–	–	–	–	–	–	–	–	–	±	–	±	±	±	±	+	+
	Tuberkelbildung: Kalkablagerung	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+

Ammerkungen: ± ein wenig, + mittelmassig, ++ ziemlich, +++ hochgradig

gewebes tritt eine Stunde nach der Injektion ein. Sie wird bei der Injektion von 10 mg Bazillen bis zum ungefähr 13 Tage beobachtet, während sie bei der von 1 mg Bazillen bis zum ungefähr 3 Tage besteht.

2) Degeneration der Leberzellen. Die Leberzellen schwellen schon eine Stunde nach der Bazilleninjektion an und ihre Färbbarkeit wird schwächer. Nach 6 Stunden entstehen Vakuolen im Kern und tritt eine ziemlich hochgradige Degeneration ein, welche Erscheinung aber transitorisch zu sein scheint, weil sie nach 72 Stunden und später nicht mehr wahrgenommen wird; nur bei einem der Fälle, die 10 mg Bazillen bekamen, tritt die Nekrose nach 13 Tagen deutlich auf.

3) Lokalisierte Zelleninfiltration. Sechs Stunden nach der Injektion tritt schon die Zelleninfiltration in dem Lobulus und der Glissonschen Scheide ein und vermehrt sich nach 8–10 Tagen. In der Glissonschen Scheide besteht sie anfänglich vorwiegend bald aus Leukozyten, bald aus runden Zellen, welche wie Histiozyten aussehen, im Lobulus aber hauptsächlich aus zusammengezogenen Sternzellen. Nach 24

Stunden—einer Woche bilden sich die Epitheloidzellen, nach einer Woche werden auch Riesenzellen gemischt gefunden und verschwinden fast alle Leukozyten. Nach 13 Tagen und später wird die Nekrose sichtbar, was aber nur selten der Fall ist.

4) Gallengangentzündung. Bei den Fällen, welche 1 mg Tuberkelbazillen bekamen, verfällt nach 24 Stunden ein Teil der Epithelien des Gallengangs der Nekrose oder Nekrobiose, und die Epithelien an der betreffenden Stelle infiltrieren die Leukozyten und andere Zellen. Nach einer Woche tritt die Gallengangentzündung etwas hervor. Auch nach 2 Wochen wird eine geronnene Substanz im Gallengang gefunden. Bei den Fällen, die 10 mg Bazillen gewonnen, wird nach 3 Stunden, 7, 11, 14 und 16 Tagen eine geronnene Substanz im Gallengang gefunden, und nach 8 und 13 Tagen werden der Abfall und die Nekrose der Epithelien des Gallengangs beobachtet.

5) Verbreitung der Tuberkelbazillen in der Leber, insbesondere ihr Auftreten im Gallengang.

Schon eine Stunde nach der Injektion erscheinen die Tuberkelbazillen in intralobulären Kapillaren, interlobulären Venen und Gallengangepithelzellen und werden besonders von den Sternzellen lebhaft gefressen. Zugleich werden sie bei der Injektion von 1 mg Bazillen nach 6 Stunden und 14 Tagen in der geronnenen Substanz und bei der von 10 mg Bazillen

TABELLE II.

Verbreitung der Tuberkelbazillen in der Leber und Resultate der Kultur aus der Galle.

Kaninchen Nr.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Inokulierte Dosis		1 mg																	
Infektionsdauer		3 st	〃	6	〃	24	〃	2 T	〃	3	〃	7	7	10	14	〃	21	28	35
Bazillenzahl in	Blutkapillaren	3.0	4.0	2.0	2.0	3.0	0.5	3.0	2.0	1.0	1.0	1.0	2.0	0.5	0.2	0.2	0.1	0.3	0
	kleinen Gefässen	12	6.0	1.0	5.0	2.0	0	1.0	2.0	1.5	0	2.0	0	1.0	0.3	1.0	1.0	0.2	0.5
	Sternzellen	4	5.5	6	4	10	8	7	18	8	10	4	11	9	6	3	4	1	3
	Gallengangepithelien	1.0	0.2	1.0	0	6.0	2.0	5	2.0	1.0	5.0	0.7	0		1.5	0.2	3.0	1.0	1.0
	Tuberkel					2.0		3.0	12.0		30.0		20.0	12.0			48.0		10.0
	Gallenkapillaren															±			
	Gallengang in Zellen	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Gallengang in geronnener Substanz	–	–	–	–	–	–	–	–	–	–	–	–	–	–	±	–	–	–
Resultat der Kultur aus der Galle		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

TABELLE II.

(Fortgesetzt)

Kaninchen Nr.			19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37
Inokulierte Dosis			10 mg																		
Infektionsdauer			1 st	3	6	24	2 T	3	7	8	10	11	13	14	16	21	28	34	41	41	44
Bazillenzahl in	Blutkapillaren		4	5	10	6	4	5	5	6	0.5	0.5	4	1	1	1	0.2	0.5	0.5	0.1	0.1
	kleinen Gefässen		3	3	3	1	1	2	9	3	7	1	3	1	2	1	1	0	0.3	2	2
	Sternzellen		10	10	8	19	14	11	11	11	8	5	6	6	6	5	5	2	3	2	1
	Gallengang-epithelien		0.6	2	2	3	0.5	2.5	10	7	4	4	10	2	4	2	4	4	1	5	3
	Tuberkel							7	10	20	4	30	cs	6	6	10	30	3	12	15	cs
	Gallenkapillaren			±				±													
	Gallengang	in Zellen	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
		in geronnener Substanz	–	±	–	–	–	–	±	±	±	±	±	±	±	–	–	–	–	–	–
Resultat der Kultur aus der Galle			–	–	–	–	–	1/2	50/2	76/2	3/2	–	–	2/2	–	–	–	–	–	–	–

Anmerkungen. Ziffer: Zahl der Tubelkelbazillen in einem Gesichtsfeld, cs: massenhaft, ±: ein wenig, Bruchzahl { Zähler: Zahl der Kolonien / Nenner: Zahl der Nährboden

nach 3 Stunden, 7, 8, 10, 11, 14 und 16 Tagen wenn auch in einer geringen Menge im interlobulären Gallengang nachgewiesen, später werden jedoch keine mehr nachgewiesen. Während sie bei dem Fall am 8 Tage nach der Injektion von degenerierten und nekrotisierten Gallengangepithelien stammen, leigen sie bei den übrigen Fällen in der geronnenen Substanz. In den Gallenkapillaren und zwischen den Leberzellen werden Stäbchen nach 3 Stunden, 3 Tagen nach der Injektion von 10 mg Tuberkelbazillen und 2 Wochen nach der von 1 mg nachgewiesen. 7 und 10 Tage nach der Injektion von 10 mg Bazillen werden zwischen den Leberzellen Histiozyten gefunden, die Tuberkelbazillen fressen.

6) Form und Schicksal der in der Leber verbreiteten Tuberkelbazillen. Die Tuberkelbazillen besetzen das Innere der Kapillaren und kleinen Blutgefässe in Form eines Stabes, und zwar liegen sie getrennt. Während sie bei der Injektion von 1 mg vom ungefähr 3 Tage an geneigt sind abzunehmen, nehmen sie bei der von 10 mg erst nach 2 Wochen ab und sind nach 5–6 Wochen noch vorhanden. In den Sternzellen sind viel granulöse Tuberkelbazillen, aber auch nicht wenig kurzstabförmige. Die Zahl der Sternzellen, die Tuberkelbazillen gefressen, zeigt 1 oder 2 Tage nach der Injektion das Maximum und scheint sich nach 10 Tagen– 2 Wochen

allmählich zu verkleinern. In den Gallengangepithelien werden bis nach 2 Wochen verhältnismässig viel Tuberkelbazillen, danach jedoch nur spärliche gefunden.

7) Kulturresultate der Galle. Bei der Injektion von 10 mg Bazillen zeigt die Kultur bei 5 Fällen (3, 7, 8, 10 und 14 Tage nach der Injektion) positive Ausfälle; diese Resultate scheinen mit denen der mikroskpoischen Untersuchungen übereinzustimmen.

8) Befunde an der Leber der Fälle, aus deren Gallengang und Galle die Tuberkelbazillen nachgewiesen worden sind.

Bei dem Fall am 14 Tage nach der Injektion von 1 mg Bazillen wird fast keine Veränderung bemerkt. 6 Stunden nach der Injektion von derselben Menge Bazillen und 3 Tage nach der von 10 mg Bazillen werden die Wucherung intralobulärer Sternzellen und die Infiltration mit den Rundzellen der Glissonschen Scheide in so leichtem Grade erkannt, dass man noch nicht von der Entstehung der tuberkulösen Veränderung sprechen kann. 7 und 16 Tage nach der Injektion von 10 mg Bazillen aber beobachtet man im Lobulus oder in der Glis sonschen Scheide die Tuberkelbildung.

4. Betrachtung.

1) Wie in der 1 Mitteilung erwähnt ist, ist die Frage, ob durch das Eindringen von Tuberkelbazillen die primäre Schädigung des Gewebes hervorgerufen wird, heute noch umstritten. Schleussing[4] fand im Lebergewebe die Bildung lokalisierter Herde, deren Färbbarkeit schwach geworden sind, und meinte, das sei die erste Veränderung durch die Tuberkelbazillen. In unseren Versuchen wurden ebenfalls im allerfrühesten Stadium die Degeneration der Leberzellen und die Hyperämie des Lebergewebes boobachtet. Aus dieser Tatsache wollen wir das Vorhandensein der primären Gewebsschädigung bejahen. Da andererseits die Tuberkelbildung verhältnismässig im frühen Stadium stattfindet, so denken wir, dass mit der primären Gewebsschädigung gleichzeitig die Wucherung der fixen Gewebszellen vor sich geht.

2) Die in den Blutstrom, namentlich in die Pfortader eingedrungenen Tuberkelbazillen werden nach Kiyono[5] teils infolge phagozytärer Wirkung der Gewebszellen, besonders der Sternzellen in der Leber gefressen und häufen sich teils in dem Sinus, dem Pfortaderast oder dem Lymphlumen um den Sinus an, ohne gefressen zu werden, und rufen dort eine tuberkulöse Veränderung hervor. Calmette und Guérin,[1] Daddi und Panà[6] und andere meinten, dass noch ein anderer Teil von den Bazillen in der Galle ausgeschieden wird. Da der Schluss der oben genannten Autoren aber auf dem Nachweis der Tuberkelbazillen aus der Galle in der Gallenblase beruht, so bleibt doch noch nicht kein Raum für das

Zweifel, ob die Bazillen wirklich aus der Leber ausgeschieden worden sind. Denn über den Prozess, durch welchen die Bakterien in der Gallenblase auftreten, gehen die Meinungen der Autoren noch auseinander; während einerseits Fütterer[7] u.a. auf der Auffassung bestehen, dass die Bakterien infolge der Wirkung der Leberzellen aus dem Blutstrom in die Galle ausgeschieden werden, wird andererseits von J. Koch[8] u.a. stark behauptet, dass sie durch Veranlassung der embolischen Entzündung der Kapillaren, die in dem Gewebe unter der Schleimhaut der Gallenblasewand vorhanden sind, direkt aus der Gallenblase ausgeschieden werden. Da es sich mit den Tuberkelbazillen ähnlich verhalten wird, würde es übereilt sein, aus dem Nachweis der Tuberkelbazillen aus der Gallenblase sofort ihre Ausscheidung aus der Leber zu schliessen.

Als wir neben dem Verbreitungszustand der Tuberkelbazillen in der Leber insbesondere beobachteten, ob die Tuberkelbazillen im interlobulären Gallengang ausgeschieden werden, stellten wir fest, dass die Tuberkelbazillen schon eine Stunde nach der Injektion in den intralobulären Kapillaren, interlobulären Venen und den Gallengangepithelien auftreten, namentlich von den Sternzellen, den histionzytären Zellen in der Glissonschen Scheide und Leukozyten gefressen werden und ferner schon nach 3–6 Stunden, meist aber nach 1–2 Wochen im Gallengang ausgeschieden werden. Diese Ergebnisse stimmen im grossen und ganzen mit denen der Kultur überein.

3) Wenn es auch keinem Zweifel mehr unterliegt, dass die Tuberkelbazillen aus dem Blutstrom in der Galle ausgeschieden werden, ist es doch heute noch nicht geklärt, durch welchen Prozess ihre Ausscheidung stattfindet. Während nämlich einerseits Calmette und Guérin,[1] Goyal,[9] Daddi und Panà[6] u.a. meinten, dass sie auf der physiologischen Ausscheidung der Leberzellen beruht, behaupteten andererseits Fränkel und Krause,[10] Rawinowitsch[2] u.a., dass sie infolge des Durchbruchs des tuberkulösen Herdes in den Gallengang hervorgerufen wird.

In unseren Versuchen ist von den Fällen, bei denen die Ausscheidung von Tuberkelbazillen in der Galle erkannt wurde, bei 3 Fällen noch keine Tuberkel in der Leber gebildet und bei 6 ist sie schon gebildet. Wenn es auch darum auf den ersten Blick scheint, als ob die Ausscheidung von Tuberkelbazillen auf dem Durchbruch des tuberkulösen Herdes beruhe, kann man dies doch verneinen, weil nach 3 wochen und später, wo der Herd noch ausgedehnter ist, die Ausscheidung von Tuberkelbazillen in der Galle nie beobachtet wird. Berücksichtigt man ferner die Tatsache, dass 3–16 Tagen, wo die Tuberkelbazillen nachgewiesen werden, verhältnismässig viel Bazillen auch in den intralobulären Kapillaren in der Leber gefunden werden, während sie danach nur sehr spärlich gefunden werden, so kann man schliessen, dass sie aus dem Blutstrom übergegangen sind.

Da in diesem Stadium auch in den Gallengangepithelien verhältnismässig viele Tuberkelbazillen gefunden werden und zwar nach 8 Tagen aus der durch Nekrose der Gallengangwand desquamierten Substanz nachgewiesen werden, wird die Ausscheidung der Tuberkelbazillen zu einem Teil durch solchen Vorgang stattfinden. Über die Frage, wie die Bakterien aus dem Blutstrom im Lobulus ausgeschieden werden, meint Kusunoki, dass es einfach durch die Klappenwirkung der Leberzellen vor sich geht, während Miyake behauptete, dass die Bazillen durch die positive Aufnahme und Ausscheidung der Sternzellen aus dem Blutgefäss in die Lymphe befördert werden und dann durch die Leberzellen in der Galle ausgeschieden werden. Diese Frage ist also heute noch nicht geklärt. Da in unseren Versuchen 14 Tagen nach der Injektion von 1 mg Tuberkelbazillen und 3 Stunden, 3 Tage nach der von 10 mg freie Tuberkelbazillen in den Gallenkapillaren und zwischen den Leberzellen nachgewiesen werden und in der Kultur aus der Galle nur ungefähr ebenso viele Kolonien wie aus dem Bronchialsekret herausgezüchtet werden, so werden die Tuberkelbazillen hauptsächlich weniger durch die positive Ausscheidung der Leberzellen, Sternzellen usw., als vielmehr aus dem Blutstrom zwischen die Zellen hindurch mechanisch mit dem strömenden Saft zusammen gehen, in die Gallenkapillaren eindringen und dann mit der Galle ausgeschieden werden. Teilweise können sie, wie wir vermuten, von den phagozytären Sternzellen befördert werden. Denn nach 7 und 10 Tagen der 10 mg Bazilleninjektion haben wir beobachtet, dass zwischen den Leberzellen Histiozyten liegen, welche Tuberkelbazillen gefressen haben.

5. Schluss.

1) Die durch Eindringen von Tuberkelbazillen in die Leber hervorgerufene pathologische Veränderung würde, wie Pagel meinte, richtig duch den Dualismus, die primäre Gewebsschädigung und die Wucherung der fixen Gewebszellen, erklärt werden.

2) Wenn die Tuberkelbazillen im Blutstrom in die Leber gelangen, so werden sie zum grössten Teil von den Sternzellen und den Histiozyten in der Glissonschen Scheide lebhaft gefressen. Ein Teil aber wird in die Galle ausgeschieden. Die Ausscheidung beginnt manchmal schon 3 Stunden nach der Injektion und dauert bis zum Ablauf von ungefähr 2 Wochen.

An dieser Stelle möchte Verf. Herrn Prof. Dr. S. Nasu in dem pathologischen Institut für seine freundliche Anleitung und Anregung den aufrichtigsten Dank aussprechen.

Diese Forschung wurde durch Überlassung von Geldmitteln des Unterrichtsministeriume für wissenschaftliche Forschung ausgeführt

wofür herzlich gedankt sei.—Prof. D. T. Kumagai und Prof. Dr. T. Ebina.

Literatur.

1) Calmette, A. et Guérin, C., C. r. Acad. Sci. Paris, 1909, **8**, No. 10, 8.
2) Rabinowitsch, L., Dtsch. med. Wschr., 1913 **39**, 103.
3) Wada, T., Igaku to Seibutugaku, 1943, **4**, 178.
4) Schleussing, H., Beitr. klin. Tbk., 1926, **63**, 317.
5) Kiyono, K. und Murakami, K., Kyoto Ig. Z., 917, **14**, 821.
6) Daddi, G. e. Panà, C., Giorn. Batter., 1938, **21**, 672.
7) Fütterer, G., Berl. klin. Wschr., 1899, **36**, 58.
8) Koch, J., Z. Hyg., 1908, **60**, 335.
9) Goyal, R. L., C. r. Soc. Biol. Paris, 1936, **123**, 379.
10) Fränkel, E. u. Krause, P., Z. Hyg., 1899, **32**, 97.

Fig. 1. Ein freier Tuberkelbazillus im interlobulären Gallengang nach Ablauf von 6 Stunden bei der Injektion von 1 mg Bazillen. (1500:1)

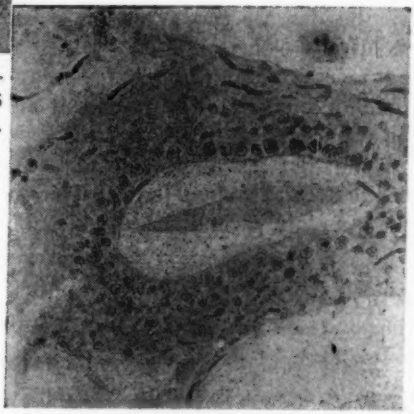


Fig. 2. Einf reier Tuberkelbazillus im interlobulären Gallengang nach Ablauf von 13 Tagen bei der Injektion von 10 mg Bazillen. (1500:1)

What is the Content of a Human Liver in Yakrinton ——Detoxicating Hormone of the Liver?

Preliminary Report.

By

Akira Sato.

(佐藤　彰)

(From the Department of Pediatrics, Faculty of Medicine, Tohoku University, Sendai. Director: Prof. A. Sato.)

(Received for publication, April 16, 1949)

Up to date we have already published more than 100 original papers on Yakrinton (the last published was 103rd report)[1]. But no report has ever been made concerning the important question: *How many units of yakrinton does a human liver contain?*

The answer is quite simple: I do not know. Why not? Because we have not studied the content in a human liver. Why not? Mainly because the organ is too small. Thus far the hormone has been extracted from the ox liver. And we do not know even what is the yakrinton-content in a single ox liver, because at least some hundree units (Rabbit-Ammonia-Units) will be required for potency testing alone.[2] And for this single purpose several livers will be necessary. There is no telling how potent a given single liver will be. Now it will be understood that a human liver, much less a part of it, is no good for the purpose.

Besides, we can never get a fresh human liver of course. A diseased liver——there will be scarcely any disease in which the organ may not be effected more or less——may contain much less yakrinton at the time of autopsy than it did at an antemortem stage; besides, it must be considered that the organ is especially prone to autolysis.

But if I am allowed to make a rough guess of the yakrinton content of the normal human (adult) liver, it may be inferred[3] that some livers may contain several dozens of Rabbit-Ammonia-Units.

However, the clinical use of yakrinton is another question. The answer will be given in another preliminary report[4]: "Is there any Case with Yakrinton-deficiency or Deficiency of the Detoxicating Liver Hormone? What is the Use of Yakrinton in Clinical Medicine?"

Literature.

1) A. Sato and T. Yoshiike, Tohoku J. Exp. Med., 1945, **50**. 2) A. Sato, (4th Report on Yakrinton) Ibid., 1927, **8**, 351. 3) A. Sato, Cf. 106th Report on the Detoxicating Hormone of the Liver (to be published in a forthcoming issue of the Journal). 4) A. Sato, to be published in a future issue of the Journal.

The Tohoku Journal of Experimental Medicine, Vol. 50, Nos. 3 & 4, 1949.

Experimentelle Untersuchungen über das Schicksal der Tuberkelbazillen im Blutstrom, insbesondere über ihre Ausscheidungswege.

Mitteilung III: Über die Verbreitung der in die Ohrvene von Kaninchen injizierten Tuberkelbazillen in der Niere, insbesondere über ihr Auftreten im Harnkanälchen der Kaninchen.

Von

Takesi Abe.
(阿 部 武)

(Aus der Medizinischen Klinik und dem Kosankinbyo Kenkyusyo der Kaiserlichen Tohoku-Universität zu Sendai, Leiter: Prof. Dr. T. Kumagai und Prof. Dr. T. Ebina.)

(Received for publication April 4, 1945)

1. Einleitung.

Foullerton und Hillier[1] haben zuerst gesagt, dass die Tuberkelbazillen aus einer nicht-tuberkulösen gesunden Niere ausgeschieden werden können. Wenn auch diese Meinung die Zustimmung vieler Autoren fand, wurde sie jedoch andererseits von sehr vielen Autoren verneint. Dimtza und Schaffhauser[2] haben soger behauptet. dass es sich schon um die Nierentuberkulose handelt, wenn die Tuberkelbazillurie nachgewiesen wird. Diese noch umstrittene Frage ist nicht zu vernachlässigen, denn ihre Klärung ist für die Diagnose der Nierentuberkulose von recht grosser Bedeutung. Wenn man aber die Versuchsmethoden der Autoren prüft, so sind sie meist nur auf den Nachweis der Tuberkelbazillen im Harn beschränkt. Um den Ausscheidungsprozess der Tuberkelbazillen zu klären, ist es noch nicht genügend; der kürzeste Weg dazu wird wohl unseres Erachtens der sein, festzustellen, ob die Tuberkelbazillen im Harnkanälchen ausgeschieden werden.

In vorliegender Arbeit wollen wir bei an Tuberkelbazillämie erkrankten Kaninchen neben dem Nachweis der Tuberkelbazillen im Harn untersuchen, ob die Tuberkelbazillen im Harnkanälchen zu finden sind.

2. Versuchsmethode und -material.

Zum Versuche wurden 37 tuberkulinnegativen gesunden Kaninchen, die in den vorhergehenden Mitteilungen gebraucht wurden, benutzt.

Die fixierten Präparate der exstirpierten Neiere wurden nach der Haematoxylin-Eosin-Färbung, der van Giesonschen Färbung und der von Katura modifizierten Tuberkelbazillenfärbung von Wada[3]) gefärbt und mikroskopiert. Bei der Sektion von den Fällen, denen 10 mg Bazillen eingesprizt wurde, entnahm man den Harn mittelst einer asepatischen Spritze durch die Punktion aus der Harnblase entnommen und nach Zusatz mit 10 ccm von 2% KaOH und 1 ccm 2.5% $ZnSO_4$ zentrifugiert. Nach tropfenweiser Hinzufügung von 2–3 ccm 4% H_2SO_4 wurde der Bodensatz auf dem Oka -Katakura schen Nährboden kultiviert.

3. Versuchsresultate.

1) Primäre Gewebsschädigung. Schon eine Stunde nach der Injektion von Tuberkelbazillen schwellen die Epithelien des Harnkanälchens, besonders des gewundenen Kanälchens und wird dann die Färbbarkeit des Protoplasmas schwach. Nach 6 Stunden findet sich eine geron-

TABELLE I.

Nierenbefund der Kaninchen mit der Bazillämie.

Kaninchen Nr.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Inokulierte Dosis		1 mg																	
Infektionsdauer		3 st	〃	6	〃	1 T	1	2	〃	3	〃	7	〃	10	14	14	21	28	35
Körpergewicht		kg 1.5	1.5	1.6	1.7	1.7	1.7	1.5	1.7	1.9	2.7	2.1	2.1	2.1	2.2	2.3	2.5	2.2	2.3
Tuberc. -Reaktion		−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+
Makroskopische Tuberkel		−	−	−	−	−	−	−	−	−	−	−	--	−	−	−	−	−	−
Mikroskopische Befund	Hyperämie	+	+	+	+	−	−	−	−	+	+	−	−	−	+	+	−	−	−
	Degeneration der Epithelien	+	+	+	+	+	+	+	±	−	−	+	−	+	−	−	−	−	+
	Exsudat	−	−	−	+	+	++		+	+	−	−	−	+	+	+	−	−	−
	Tuberkelbildung: Polym. Leukozyt.																		
	Tuberkelbildung: Endothelzellen					+	+		+				++				+	+	++
	Tuberkelbildung: Histiozyten				+														
	Tuberkelbildung: Epitheloidzellen																		−
	Tuberkelbildung: Lymphozyten																		−
	Tuberkelbildung: Riesenzellen																		−
	Tuberkelbildung: Nekrose																		−
	Tuberkelbildung: kollagene Fasern																		−

Tabelle I.

(Fortgesetzt)

Kaninchen Nr.			19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37
Inokulierte Dosis			10 mg																		
Infektionsdauer			1 st	3	6	1 T	2	3	7	8	10	11	13	14	16	21	28	34	41	41	44
Körpergewicht			1.5	1.7	1.9	1.4	2.0	1.9	2.1	1.4	2.0	1.5	2.3	1.4	2.0	2.1	1.5	1.4	2.5	22	1.5
Tuberc. -Reaktion			–	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+
Makroskopische Tuberkel			–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Mikroskopische Befund	Hyperämie		–	+	+	+	++	–	–	–	–	–	–	–	–	+	–	–	+	–	+
	Degeneration der Epithelien		+	+	+	+	++	±	±	–	–	–	–	–	–	+	–	–	–	–	–
	Exsudat		–	–	++	++	+	+	+	+	+	–	+	–	+	–	–	–	–	–	–
	Tuberkelbildung	Polym. Leukozyt.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–	–
		Endothelzellen	–	–	–	–	–	–	+	+	+	+	+	++	++						
		Histiozyten	–	–	–	–	–	–	–	+	+			–	–	–	–	–	–	–	–
		Epitheloid-zellen										+	–	+	+	+	+	+	+	+	+
		Lymphozyten								+	+			+	+	+	+	+	+	+	+
		Riesenzellen												–	–	–	–	–	–	–	–
		Nekrose												–	–	+	+	+	+	+	+
		kollagene Fasern												–	–	–	–	–	+	+	+

Anmerkungen: ± ein wenig, + mittelmässig, ++ ziemlich, +++ hochgradig.

nene Substanz in dem Harnkänälchen und der Bowmannschen Kapsel. Nach 2 Tagen erfolgt manchmal eine glastropfenweise Entartung der Epithelien. Ferner tritt die Hyperämie des Gewebes schon im Frühstadium auf.

2) Verbreitung der Tuberkelbazillen in der Niere, besonders ihr Ausscheidung. Was den Verbreitungszustand der Tuberkelbazillen der Niere anbelangt, erscheinen die Tuberkelbazillen nach Ablauf von einer Stunde nach der Injektion im Glomerulus, in den Kapillaren des Interstitiums und in den Zellen des Interstitiums und bald danach in geronnener Substanz in der Bowmannschen Kapsel und dem Harnkanälchen ausgeschieden. Sie werden nämlich bei der Injektion von 1 mg Bazillen nach 48 Stunden in der Bowmannschen Kapsel und nach 24, 48 Stunden, 10 Tagen im Harnkanälchen und bei der von 10 mg Bazillen nach 10 Tagen in der Bowmannschen Kapsel und nach 3,

Tabelle II.

Verbreitung der Tuberkelbazillen in der Niere und Resultate der Kultur aus dem Harn.

Kaninchen Nr.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Inokulierte Dosis		1 mg																	
Infektionsdauer		3 st	"	6	"	24	"	2 T	"	3 T	"	7	7	10	14	14	21	28	35
Bazillenmenge in	Kapillaren	3	1	2	1	3	2	2	1	1	0.5	1	0.5	0.2	0.3	0.1	0.2	0.1	0.1
	Glomerulus	11	2	8	6	6	16	4	4	6		5	7	3	5	6	8	3	0.7
	Zellen des Interstitiums	5	2	4	5	6	3	4	3.5	4	5	4	4	3	2	1	3	1	0
	Zellinfiltration																	—	2
	Bowmannsche Kaps.	—	—	—	—	—	—	±	—	—	—	—	—	—	—	—	—	—	—
	Harnkanälchen	—	—	—	—	—	±	±	—	±	—	—	—	±	—	—	—	—	—
Kulturresultat aus dem Harn		Nicht ausgeführt																	

Tabelle II.

(*Fortgesetzt*)

Kaninchen Nr.		19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37
Inokulierte Dosis		10 mg																		
Infektionsdauer		1 st	3	6	24	2 T	3	7	8	10	11	13	14	16	21	28	34	41	41	44
Bazillenmenge in	Kapillaren	4	3	6	2	4	3	5	5	3	1	2	0.5	0.4	0,5	0.2	0.1	0.2	0.1	0.1
	Glomerulus	5	3	4.5	6	20	5	2	6,5	7	2,5	4.5	4	3	3.3	2.5	5.5	3.5	4	4
	Zellen des Interstitiums	4.5	5	5	6	6	6	3	6	7	3	4.5	3	2	4	2	2	1	1	1
	Zellinfiltration										6		16	11	cs	7	cs	30	cs	10
	Bowmannsche Kaps.	—	—	—	—	—	—	±	±	±	—	—	—	—	—	—	—	—	—	—
	Harnkanälchen	—	—	—	±	—	—	±	±	±	—	±	—	±	—	—	—	—	—	—
Kulturresultat aus dem Harn		—	—	—	—	—	—	23/2	2/2	125/2	8/2	110/2	2/2	3/2	—	—	—	—	—	—

Anmerkungen. Ziffer: Zahl der Tnberkelbazillen in einem Gesichtsfeld, cs: massenhaft, ±: ein wenig, Bruchzahl: Zähler, zahl der Kolonien; Nenmer, zahl der Nährboden

24 Stunden, 7, 8, 10 13 und 16 Tagen im Harnkanälchen ausgeschieden. In den Kapillaren nehmen die Tuberkelbazillen nach ungefähr 1–2 Wochen ab.

3) Beziehung zwischen dem Auftreten der Tuberkelbazillen in dem Harnkanälchen und der Bowmannschen Kapsel und dem histologischen Bild. Bei 8 Versuchstieren (24, 48 Stunden, 3, 10 Tage nach der Injektion von 1 mg Bazillen, 3 Stunden, 1, 7, 13 Tage nach der von 10 mg Bazillen) wird kein tuberkulöser Herd wahrgenommen, wenn auch bei ihnen die Tuberkelbazillen ausgeschieden werden. Bei 2 Fällen (8 und 10 Tage nach der Injektion von 10 mg Bazillen) tritt nur leichte Zellinfiltration auf. Bei einem (16 Tage nach der Injektion von 10 mg Bazillen) bildet sich die Tuberkel. Bei 2 Tieren (7 und 10 Tage nach der von 10 mg Bazillen) werden Tuberkelbazillen (Stäbchen) zwischen den Epithelien des Harnkanälchens nachgewiesen.

4) Resultate der Kultur der Tuberkelbazillen im Harn. Bei 7 Fällen (7, 8, 10, 11, 13, 14 und 16 Tage nach der Injektion von 10 mg Bazillen) fällt die Kultur der Tuberkelbazillen im Harn positiv aus; bei 5 davon stimmen die Resultate mit denen der histologischen Nachweisung überein.

4. Betrachtung.

1) Aus unseren Versuchen an der Niere ging hervor, dass schon im Frühstadium mit der Hyperämie des Gewebes deutlich die Degeneration der Epithelien der Bowmannschen Kapsel und derjenigen des Harnkanälchens, besonders des gewundenen Kanälchens herbeigeführt wird und gleichzeitig die Zunahme der Blutkapillarenendothelzellen und der Kerne des Glomerulus vor sich geht. Wir glauben darum, dass, wie in der 1. und 2. Mitteilung erwähnt ist, der Dualismus von Pagel[4] auch für die Niere gilt.

2) Über Auftreten der Tuberkelbazillen im Harnkanälchen. Über die Verbreitung der in den Blutstrom eingedrungenen Tuberkelbazillen in der Niere gibt es überhaupt zwei Theorien. Während Baumgarten, Zondeck[5] und andere meinten, dass die Tuberkelbazillen in den Kapillaren der Rinde und des Markes stocken und hier eine pathologische Veränderung hervorrufen, behaupteten Orth[6] und andere, dass die Tuberkelbazillen durch den Nierenglomerulus im Harnkanälchen ausgeschieden werden, dem Markharnkanälchen anhaften und hier die Marktuberkulose herbeiführen. Nach Eberbach[7] u.a. stocken die Tuberkelbazillen in dem Glomerulus wie in den Markblutkapillaren, werden anderseits auch in Harnkanälchen ausgeschieden und verursachen nach verschiedenen Momenten, der Widerstandskraft des Rind- und Markgewebes der Niere, dem Virulenz der Tuberkelbazillen, der Bazillenmenge usw., die Mark- oder Rindetuberkulose. Die Ausscheidung der Tuberkelbazillen im Harn wird von Dimtza und Schaffhauser[2] u.a. verneint, aber von Foullerton und Hillier und Deist[8] u.a

bejaht. Die Auffassungen beruhen aber fast alle nur dem Vergleich der Tuberkelbazillen im Harn mit den Nierenbefunden, und es ist fast kein Versuch angestellt worden, das Vorhandensein der Tuberkelbazillen in Harnkanälchen oder der Bowmannschen Kapsel festzustellen und die Nierenbefunde damit zu vergleichen. Darum würde es noch übereilt sein, sofort, zu entscheiden, ob die Tuberkelbazillen die nicht-tuberkulöse Niere passieren.

In unseren Versuchen haben wir festgestellt, dass bald nach der Bazilleninjektion viele Tuberkelbazillen in den Rinde- und Markkapillaren, nicht zu sprechen von dem Glomerulus, vorhanden sind, später hier herum die Tuberkeln entstehen und zugleich Bazillen in sehr geringer Menge bis zum Ablauf von 2 Wochen, wo verhältnismässig viel Tuberkelbazillen im Blutstrom vorhanden sind, (frühestens 3 Stunden und spätestens nach 16 Tagen nach der Injektion) in geronnenen Substanzen in der Bowmannschen Kapsel oder dem Harnkanälchen frei liegen. Als wir untersuchten, ob in ihrer Umgebung die pathologische Veränderung an der Niere ist, wurde bei 8 Fällen keine tuberkulöse Veränderung erkannt, während bei 2 leichtgradiger Infiltration und bei einem Fall nur eine Tuberkel konstatiert wurde. Wenn auch bei dem letzten Fall die Tuberkelbazillen auf den ersten Blick infolge der tuberkulösen Veränderung entstanden zu sein scheinen, kann man doch unseres Erachtens bei diesem Befunde nicht viel Gewicht auf das Vorhandensein der Tuberkel legen, denn bei Fällen mit verhältnismässig fortgeschrittenen Veränderungen wurde die Ausscheidung von Bazillen nicht beobachtet. Da aber die frei liegenden Tuberkelbazillen fast immer in der geronnenen Substanz gefunden werden, sind sie wahrscheinlich infolge der primären Schädigung des Gewebes mit Saft zusammen ausgeschieden. Die Tuberkelbazillen im Harnkanälchen werden wahrscheinlich zum Teil aus der Bowmannschen Kapsel, zum Teil aus dem Harnkanälchen ausgeschieden; denn 7 und 11 Tage nach der Injektion von 10 mg Bazillen wurden Tuberkelbazillen zwischen den Epithelien des Harnkanälchens gefunden.

3) Die Ausscheidungsmenge der Bazillen im Harn ist auch in den Kulturbefunden sehr spärlich, sie scheint nämlich nicht verschieden von der aus den anderen Organen. Darum glauben wir, dass auch die Ausscheidung von Tuberkelbazillen aus der Niere gewissermassen mechanisch stattfindet und schwer als eine positive physiologische Wirkung anzusehen ist.

5. Schluss.

Die Tuberkelbazillen im Blutstrom werden in den Rinde- und Markkapillaren, nicht zu sprechen von dem Glomerulus, verbreitet und bilden hier herum die Tuberkeln. Zugleich werden sie zum Teil aus der

Glomerulusschlinge und dem Harnkanälchen ausgeschieden und treten im Harn auf. Die Ausscheidung dauert ungefähr von der 3 Stunde bis 16 Tage nach der Bazilleninjektion.

An dieser Stelle möchte Verf. Herrn Porf. Dr. S. Nasu in dem pathologischen Institut für seine freundliche Anleitung und Anregung den aufrichtigsten Dank aussprechen.

Diese Forschung wurde durch Überlassung von Geldmitteln des Unterrichtsministeriums für wissenschaftliche Forschung ausgeführt, wofür herzlich gedankt sei. Prof. Dr. T. Kumagai und Prof. Dr. T. Ebina.

Literatur.

1) Foulerton und Hillier, zit. nach Kekkaku, 1940, **18**, 896.
2) Dimtza, A. and Schaffhauser, F., Z. urol. Chir., 1932, **35**, 440.
3) Wada, T., Igaku to Seibutsugaku, 1943, **4**, 178.
4) Pagel, W., Beitr. klin. Tbk., 1925, **61**, 221.
5) Zondeck, M., Z. Urol., 1920, **14**, 288.
6) Orth, J., B. K.. W., 1912, **49**, 1062.
7) Eberbach, C. W., J. of Urol., 1927, **17**, 233.
8) Deist, H., Kl. W., 1933, **1**, 26.

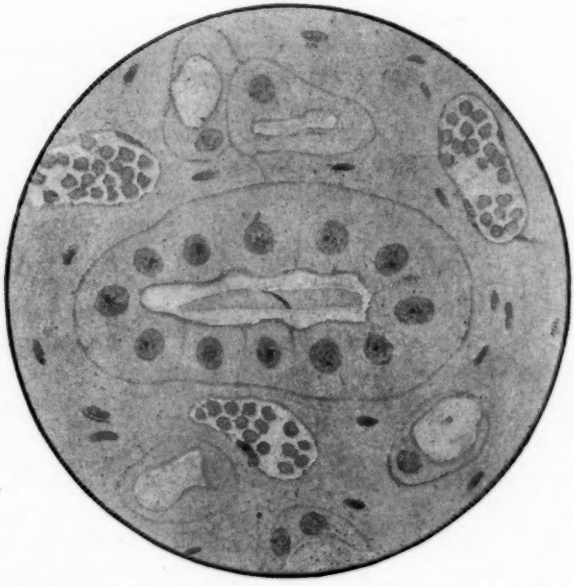


Fig. 1. Ein freier Tuberkelbazillus im Harnkanälchen nach Ablauf von 24 Stunden bei der Injektion von 1 mg Bazillen. (1500:1)

Fig. 2. Ein freier Tuberkelbazillus im Harnkanälchen nach Ablauf von 8 Tagen bei der Injektion von 10 mg Bazillen. (1500:1)

The Tohoku Journal of Experimental Medicine, Vol. 50, Nos. 3 & 4, 1949.

Electroencephalograms and Conditioned Reflexes.

By

Kōiti Motokawa and Bun'iti Huzimori.

(本川 弘一) (藤森 聞一)

(From the Physiological Laboratory of Prof. K. Motokawa, Tohoku University, Sendai.)

(Received for publication, April 15, 1945)

The conditioned reflexes have offered one of the most important methods which enabled us to study objectively psycho-nervous mechanisms in the higher centers of the brain of man and animals. The theory of Pavlov[1] on the central mechanism of conditioned reflexes is based upon a number of experimental facts so well-established that nothing more seems to be necessary to add. But in view of the fact that the theory is based merely upon the findings at peripheral organs such as salivary glands or muscles and not at all upon the direct observation of the phenomena occurring in the brain itself, it is evident that the theory is still open to criticism.

On the other hand, the electrophysiology of the central nervous system has made remarkable progress in recent years, furnishing us with an excellent means for the direct observation of the brain function. The theory of conditioned reflexes, therefore, has to be criticized above all from an electrophysiological point of view. It seems rather strange that little efforts should have been made to connect these important fields of the brain physiology with each other. It is, therefore, the purpose of the present study to investigate the changes of brain waves accompanying conditioned reflexes in man (galvanic skin-reflex) in order to fill up the above mentioned gap in our knowledge. In this filed of researches, the only work available is that of Loomis, Harvey and Hobart[2]. They showed that the depression of α-waves could be conditioned, but did not refer to the mechanism of conditioned reflexes.

Method.

The brain potentials were recorded from various parts of the scalp, e.g. from the occiput, vertex, forehead, etc. with a diffuse lead on the earlobe and a different lead on the part to be tested. The electrodes were flattened pellets of silver, about 3 mm. in diameter, fixed to the

scalp with electrode-paste and court-plaster. The recording instrument consisted of a four stage resistance-capacity amplifier and an oscillograph of the electro-magnetic type.

The subject, with his eyes closed, reclined on a bed in a darkened, quiet, shielded room, while the recording and all stimulating procedures were carried out in an adjoining room lest the experiment should be disturbed by the extra-stimuli which might be caused by these procedures.

In the circuit of the galvanometer for recording galvanic skin-responses (gsr.) there was a blocking condenser of 40 μF which served to avoid troublesome shifts of the base line.

Result.

The changes in eeg. of man elicited by internal or external stimuli may be classified roughly into the following three kinds of pattern, namely: 1. the diminution of α-waves, 2. the augmentation of β-waves and 3. irregular deflections of the base line. The first and second kind of pattern do not fail to appear more or less markedly whenever the subject is under excited conditions, so that they are generally regarded as a sign of cortical excitation. The third, however, has received only scant attention, partly because it does not always appear and partly because it is somewhat difficult to distinguish it from artefacts due to bodily movements. We are sure that the deflections of the base line observed in our cases beared little on bodily movements, and that they were related to strong mental excitement.

Since all these three kinds of potential changes are indications of the excited state of the brain, we may express them with a common term " excitation potential (Ep.)".

1. *Changes in Brain Potentials in the Course of the Establishment and in the Extinction of Conditioned Reflexes.*

In the following investigation a faradic current of about 0.5 seconds in duration from Porter's inductorium was used as an unconditioned stimulus, the strength of the current being so chosen that it caused only paresthetic sensation but no pain. Metallic electrodes about 3 mm. apart were placed on the foot of the subject and fixed with a bandage, the electric contact being secured by electrode-paste. As a conditioned stimulus served an electric bell, which was rung within the recording room, so that the sound of the bell, much reduced in strength, reached the subject.

This weak stimulus was usually little effective on eeg. as well as gsr., unless it caused special mental processes such as astonishment, unease, etc. However, the electrical stimulus was always so effective on gsr. that it could be used as unconditioned stimulus.

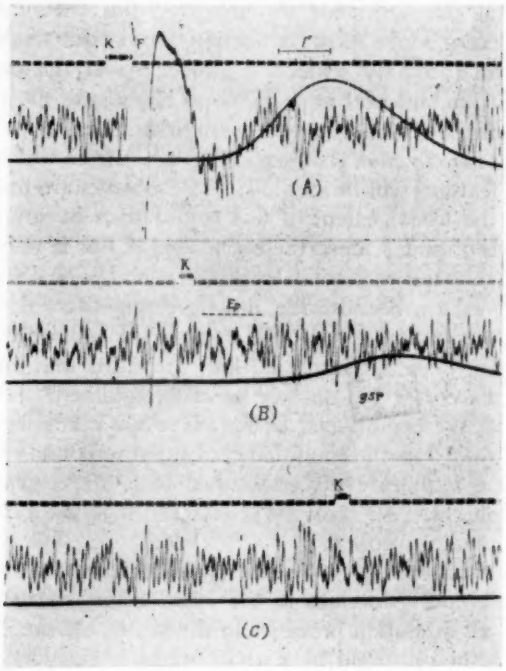


Fig. 1. Relation between brain waves and galvanic skin- responses in man.
A: Effect of stimulation by an electric bell (K) associated with a faradic current (F) before conditioning.
B: Effect of K alone after conditioning. "Excitation potential" (Ep.) precedes a conditioned galvanic skin- response (gsr.).
C: After extinction no skin- response appears upon K, but a trace of Ep. is still perceived.

The effect of the acoustic stimulus, K followed by the electric F is shown in Fig. 1 (A). The remarkable excursion of the base line is an artefact due to the induction current. If these combined stimuli repeated several times with an interval of about 3–4 minutes, a conditioned reflex is established. An example of the so established conditioned reflexes is illustrated in Fig. 1 (B). As seen from this figure, the conditioned stimulus K alone, which had been little effective before conditioning, caused a brief depression of the α-waves (Ep.) and a galvanic skin-reflex (gsr.). The latency of the Ep. is about 0.3 sec., and that of the gsr. about 2 sec. This marked difference in latency is chiefly due to the slowness of the skin-response.

Conditioned reflexes become weaker and weaker, and at last disappear completely, if the conditioned stimulus is repeatedly given without ac-

companying the unconditioned stimulus, and this phenomenon is called experimental extinction. As the extinction must be due to some physiological changes produced by extinction procedure in the central nervous system, it is of fundamental importance to investigate the changes in the pattern of brain potentials under this condition.

The result of extinction is shown in Fig. 1 (C), from which the following characteristic features will be seen: 1. The *a*-waves are more completely developed in the whole extent of this record than in any other records, 2. no gsr. is seen, and 3. nevertheless, a sign of Ep. is visible. The first fact indicates that the excitation level of the cortex has been lowered by extinction procedure, because the full development of the *a*-waves is a sign of a low excitation level. The second shows that the conditioned reflex has really been extinguished, and the third that the conditioned excitation of the cortex has not yet been extinguished.

In general, the conditioned excitation of the cortex resists extinction more strongly than does the conditioned external response. On the contrary, conditioning is more easily established with the central process than with the peripheral. All these facts indicate that the external response is merely a secondary process.

Pavlov assumes that a certain physiological process designated as " internal inhibition " develops in the brain during extinction, and that it acts against all excitation processes in the brain. If the internal inhibition happens to be disturbed by a strong stimulus and to loose its control over the excitation, the conditioned reflex once lost sight of appear again, though momentarily, and this phenomenon is called " disinhibition."

In the course of extinction, it was occasionally observed in our experiment that the once reduced conditioned responses showed a temporary recovery without any appreciable external cause, and this state of affairs gave the results of our experiment a somewhat unreliable appearance, but the survey of the eeg. taken at that moment revealed the very cause of the accidental recovery of the conditioned responses; in the eeg. a slight, but diffuse excitation pattern prevailed, showing that in spite of the absence of external stimuli, some excitation must have happened in the brain. The accidental internal excitation must have disrupted the established internal inhibition and have led to the recovery of the conditioned reflex. The above mentioned supernormal development of the *a*-waves in the extinction can be interpreted also in the light of Pavlov's theory, for very active *a*-waves correspond, in general, to the absence of excitation in the cortex or to the dominance of internal inhibition in the terms of Pavlov. He empasizes the positive nature of inhibition, persuading us with many experimental facts that the inhibition does not mean the mere absence of excitation, but implies a positive suppression

of central excitation. The fact that the α-activity is raised in extinction to such a high level as is hardly attainable under normal conditions provides another strong support of Pavlov's view, because some positive action seems to be necessary to put the brain into such a supernormal state.

2. *Delayed Conditioned Reflexes.*

When the interval between the beginning of the conditioned stimulus and the administration of the unconditioned stimulus is long, the conditioned response does not take place until an appreciable time after the beginning of the conditioned stimulus, but sometime before its termination. Such reflexes are known as delayed conditioned reflexes. We tried to establish this sort of reflexes with the same pair of stimuli as used in the previous experiment. We found in this investigation that the acoustic stimulus which had been taken for always ineffective in the previous experiment could give rise to a fairly strong unconditioned gsr. in a certain stage of conditioning. This relation was lost sight of in the above experiment, probably because both sorts of responses, unconditioned and conditioned could hardly be distinguished from each other, but in the experiment on delayed conditioned reflexes they appeared separately in such a manner that the unconditioned response appeared with a constant latency of about 2 seconds, while the conditioned one apeared more or less delayed. These circumstances enabled us to study the behavior of both sorts of responses in the course of conditioning. In this experiment, the unconditioned stimulus was given 10 seconds after the beginning of the conditioned stimulus. The record shown in Fig. 2 (A) was taken in a stage of conditioning in which the establishment of the conditioned reflex was not yet complete. As seen from this figure, both responses, unconditioned and conditioned do not differ in magnitude at least in this stage of conditioning, but both sorts of Ep. (the 1. and 2. Ep.) are generally different in their duration. This difference in the behavior of the external and internal responses indicates that the external response is not always a faithful image of the internal process.

As the conditioning was further strengthened by repetition of the combined stimuli, the unconditioned skin-response became weaker and at last disappeared completely. On the contrary, the conditioned response increased in magnitude, as if it had developed at the cost of the unconditioned response.

The behavior of eeg. is more interesting. The unconditioned Ep. ceased to appear in a much earlier stage of conditioning, in which the unconditioned skin-response was still clearly seen. The conditioned Ep. took a very protracted course, beginning about 2 seconds before the onset of the delayed conditioned skin-response and terminating toward the end

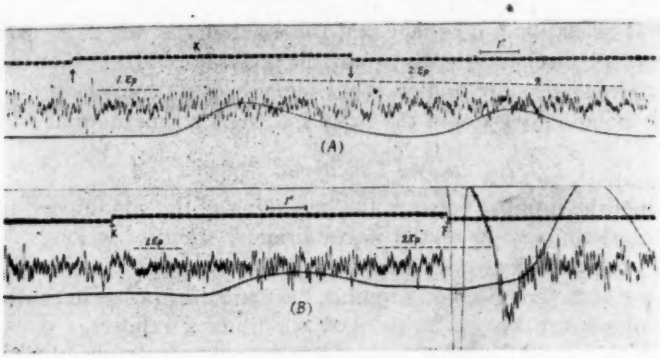


Fig. 2. Delayed conditioned reflexes. The interval between the beginning of the conditioned stimulus (K) and the administration of the unconditioned stimulus (F) was 10 seconds.

A: When conditioning is not complete there appear two kinds of skin- responses of which the one is delayed and the other not. The delayed Ep. is less continuous and of longer duration, as suggested by a long broken line.

B: Under the same condition as A. The unconditioned stimulus (F) was delivered about 8.7 seconds after the beginning of the conditioned stimulus (K). Upon F the Ep. came immediately to an end and regular α-waves appeared (temporal induction).

of the latter, as indicated by a long broken horizontal line in the Fig. The pattern is not homogeneous throughout the whole course of the Ep.; on the background of usual excitation pattern short trains of fairly regular *a*-waves appear from time to time, so that the pattern presents, as a whole, a discontinuous appearance. This characteristic feature of the eeg. seems to throw some light upon the nature of the delayed conditioned reflex.

As to the mechanism of delayed conditioned reflexes, Pavlov advanced the hypothesis that the delay is due to internal inhibition which suppresses the development of the excitation underlying the conditioned response. In the light of this hypothesis, the regular sequence of *a*-waves seen in the latency of the conditioned response may be interpreted as an expression of prevailing internal inhibition, and the alternation of active and inactive *a*-phases in the subsequent stage (the inhomogeneous pattern of eeg.) must be the indication of the rivalry between inhibition and excitation. The pattern of Ep. shows in this manner very clearly that the excitation which underlies the conditioned response can get over the internal inhibition after a fairly long rivalry and so gives rise to the response only after a considerable latency.

3. *Induction.*

The delayed conditioned Ep. appeared after a latency of about 5–7 seconds and lasted for about 10 seconds unless the unconditioned electric

stimulus was delivered at the 10th second from the beginning of the conditioned acoustic stimulus. When the unconditioned stimulus was given, the Ep. soon came to an end without taking the protracted course characteristic of the delayed conditioned reflex, as illustrated in Fig. 2 (B). One might suppose that the unconditioned stimulus would reinforce the Ep. and prolong its course, when the stimulus is delivered during the excitation, but in reality, the reverse is the case; in the case represented in this figure, the Ep. is only about 3 seconds in duration, and this is very short compared with that of the Ep. shown in Fig. 2 (A) in which no unconditioned stimulus was given.

This curious fact will find its explanation in the property known as "induction" of the central nervous system. As pointed out by Sherrington, Pavlov and others, any process evoked in the central nervous system by an external cause is in general followed by another process quite reversed in quality (temporal induction), and when a process is taking place in a certain region of the central nervous system an opposite effect is induced in the immediate neighborhood of that part (spatial induction). Although the mechanism of induction has not yet been elucidated there are many experimental facts which can be interpreted only in terms of induction. As to our experimental fact mentioned above, the following interpretation seems legitimate: The momentary strong excitation caused by the unconditioned stimulus is followed by an opposite effect, i.e. inhibition which puts the prevailing Ep. immediately to an end and leads to the recovery of α-activity.

Discussion.

The fundamental assumption in the study of conditioned reflexes is that responses observed at peripheral organs reflect faithfully the central processes involved, and our investigation on eeg., direct indicator of the cortical function has corroborated that the assumption is valid at least approximately. We have, however, sometimes observed that a conditioned Ep. was not followed by a perceivable galvanic skin-response, especially in the early stage of conditioning and in some phase of extinction, and this very fact seems to be sufficient to show that the fundamental assumption has only approximate validity. It might, however, be argued that the failure of strict parallelism between both phenomena could be due to the difference in sensibility of the instruments for both sorts of phenomena, but such argument is hardly tenable, because the discrepancy still existed even when the sensibility of the recording apparatus for the galvanic skin-response was raised so high that a conditioned skin-response of ordinary magnitude would overshoot the upper margin of the recording paper.

The most probable explanation of this fact is that the central excitation must surpass a certain threshold to be able to set up impulses in the nerve supplying the effector organ, so that sub-threshold conditioned excitation, though it can be detected by means of electroencephalography, cannot elicit any peripheral response at all.

In the reflexology, excitation and inhibition are the most important principles on which the explanation of various aspects of conditioned reflexes are based. They are to the reflexology what positive and negative charge are to the science of electricity.

The question now arises as to whether excitation and inhibition are different things opposite in nature, or whether they are nothing but expressions for quantitative difference of one and the same thing, and it seems difficult to answer this question so long as we confine ourselves to investigations with peripheral responses as indicator. Our investigation has revealed that inhibition exhibits no special positive form of potential pattern, showing itself only in the absence of Ep. It seems, therefore, that excitation and inhibition are nothing but conventional terms convenient for denoting the excitation level of the brain. Our experiment, however, is not conclusive enough to rule out all the possibilities of dualism, for some inhibition pattern of concrete form might exist in a certain part of the brain hardly accessible to the usual electroencephalography.

Summary.

Conditioned galvanic skin-reflexes were established in man with an electric bell as a conditioned stimulus and with a brief faradic current as an unconditioned stimulus.

1. Upon the conditioned stimulus there appeared characteristic changes in eeg. (Ep.), which were followed by a galvanic skin response. When conditioning was not complete, only an Ep. appeared without being followed by any skin-response.

2. The skin-response ceased to appear, but the Ep. did not disappear completely by extinction procedure (repetition of conditioned stimuli). These facts suggest that the Ep. is a precursor for the external response.

3. When the interval between the beginning of the conditioned stimulus and the administration of the unconditioned stimulus was long the appearance of the Ep. as well as that of the skin-response were delayed (delayed conditioned reflex). The potential pattern under this condition is discontinuous and of long duration, and this characteristic feature can be explained by the assumption that the delayed conditioned response appears after a long rivalry between excitation and inhibition.

4. The long lasting Ep. in the delayed conditioned reflex came immediately to an end when the unconditioned stimulus was delivered.

This phenomenon is due to " temporal induction " in the cortex.

References.

(1) Pavlov, I. P., Conditioned reflexes. An investigation of the physiological activity of the cerebral cortex. Trans. by G. V. Anrep. London, Oxford Univ. Press, 1927.

(2) Loomis, L., E. Harvey and G. Hobart, J. Exp. Psychol., 1936, **19**, 249.

Monocytic Croup; Probably the First Case ever Published.*

By

Akira Sato, Kazuo Tachibana and **Jun-ichi Ishikawa.**

(佐藤　彰)　(立花一夫)　(石川淳一)

(From Department of Pediatrics, Faculty of Medicine, Tohoku University, Sendai. Director: Prof. A. Sato.)

(Received for publication, April 16, 1949)

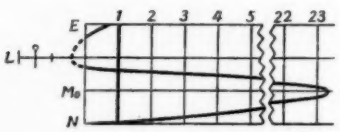
Cases of monocytic angina are too numerous to enumerate, while there is no case reported of "monocytic croup," so that we are rather surprised at the penetrative and instructive description by Wintrobe[1] to the effect that *laryngeal obstruction is unknown.*

Case Report.

A. H., a girl aged 22 months, was admitted to our Pediatric Clinic on Oct. 31, 1945 with complaints of high fever, *stridor* and *aphonia*. On the presumptive diagnosis: "Diphtheric Croup" 3,000 units of antitoxin were injected intramuscularly, even though diphtheria bacilli had not been identified. The next day 4,000 units were given again. Laryngoscopic examination on Nov. 2 showed a reddened and edematous larynx without any membrane; no diphtheria bacilli on culture. Temperature was nearly 40°C on Nov. 1, 38°C on the next day; then it was irregular, though mostly not feverish. The girl, almost recovered, was discharged after 2 weeks' stay.

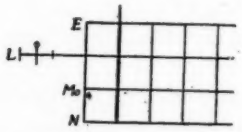
Blood will be shown by way of ELMoNogram (Fig. 1 and Fig. 2)[2]; monocyte count was ca. 9400 (white count was 12,000) on Nov. 2.

Fig. 1.



Elmonogram of our case

Fig. 2.



Elmonogram for 2 years of age

As shown by Longscope[3] in cases of infectious mononucleosis, a certain relation between temperature curve and absolute count of mononuclear cells was observed in our case. As to details of the course, the blood picture and the hemo-myelogram cf. 86th Hematological Paper.[4]

"Monocytic Croup" was suggested by us for the disease.

Literature.

1) M. M. Wintrobe, Clinical Hematology, 2nd Edit., Philadelphia, 1947, 803.
2) A. Sato and K. Hayashi, Tohoku J. Exp. Med., 1947, **49**, 37.
3) W. T. Longscope, Amer. J. Med. Science, 1922, **164**, 781.
4) K. Tachibana and I. Ishikawa, 84th Hematological Paper, Tohoku J. Exp. Med. to be published in a forthcoming number of this Journal.

*Read at the 32nd General Meeting of the Tohoku Medical Society, Sendai, Nov. 2, 1947.

The Tohoku Journal of Experimental Medicine, Vol. 50, Nos. 3 & 4, 1949.

Electroencephalograms of Man in the Generalization and Differentiation of Conditioned Reflexes.

By

Koiti Motokawa
(本川 弘一)
(From the Physiological Laboratory of Prof. K. Motokawa, Tohoku University, Sendai.)

(Received for publication, April 15, 1945)

In the previous communication Motokawa and Huzimori[1] reported the changes in electroencephalograms of man in the conditioned galvanic skin-reflexes which had been worked out with an electric bell and an induction current as the conditioned and the unconditioned stimulus respectively. We proposed in the communication to express all the changes in brain-waves involved in the conditioned reflex, i.e., the diminution in the amplitude of α-waves, the augmentation of β-activity and some irregular deflections of the baseline with the common term "excitation-potential (e.p.)", because they are without doubt some indication of cerebral excitation and appear almost always superimposed upon one another.

As an electric stimulus is, in general, a very effective means for eliciting a galvanic skin-response (g.s.r.) it is an easy matter to establish conditioned reflexes upon the basis of g.s.r. evoked by electrical stimulation. On the contrary, a light-stimulus is almost ineffective in this regard unless it causes particular states of mind, e.g. astonishment, embarrassment, apprehension etc. so that only a slight g.s.r., if any, can be evoked by a light-stimulus of ordinary strength. As to the effect upon the brain-waves the relation is quite reversed: the light-stimulus has the most striking effect upon the α-waves of man while the electric stimulus is in general little effective, as pointed out by Motokawa and Huzimori[2]. On account of this effectiveness the light-stimulus can be used as an unconditioned stimulus for conditioned responses of the brain-waves. Loomis, Harvey and Hobart[3] showed that an acoustic stimulus which was little effective acquired the ability to reduce or extinguish the α-waves if it was applied several times associated with an effective light-stimulus. In our previous

experiments, in which the electric stimulus was used as the unconditioned stimulus, the conditioned g.s.r. generally followed an e.p., an electric expression of physiological processes in the cortex involved in the conditioned reflex.

Now the question arises whether the conditioned e.p. observed in the experiment of Loomis *et al.* is a reflex-response of the cortex as a final event of the sequence of the reflex- processes or whether it is a precursor for some external reflex-action. If the second alternative is true, it must be followed by some external reflex-response.

In the present investigation I have confirmed that the conditioned e.p. is actually followed by a remarkable g.s.r., and this is worth noticing because the light-stimulus used as the unconditioned stimulus is little effective in regard to the g.s.r. and because it seems hardly adequate as the unconditioned stimulus for this sort of conditioned reflex.

Method and Results

1. *The Course of the Establishment of Conditioned Reflexes.*

An acoustic stimulus from a metronome (M) was used as the conditioned stimulus throughout this experiment. This sort of stimulus evoked a slight g.s.r. but almost no change in brain-waves. The electric light of the room was switched on 2 seconds after the beginning of M and switched off 2 seconds later, the duration of the unconditioned light-stimulus being thus 2 seconds. This stimulus evoked always a very marked e.p., but almost no g.s.r., as stated above.

The method of recording electroencephalograms and g.s.r. was the same as that employed in our previous experiment.[1)]

When a subject was not yet conditioned, the effect of the combination of the conditioned and unconditioned stimuli was equal to the sum of each effect, but the deviation from the sum became more and more striking as the conditioning proceeded. The time course of these changes is illustrated schematically in Fig. 1. (a) and (b) of the Fig. represent the effect of M alone and that of the light-stimulus (L) alone respectively, and (c) is the effect of the combined stimuli before conditioning. After repetitions of the combined stimuli a stage (d) was reached at which 2 g.s.r. appeared upon the combined stimuli, the first response being greater than the second. The latter increased in magnitude at the cost of the former, when the conditioning was strengthened (stage e), and after the repetition of about 15–20 times, a stage (f) was reached at which the second reponse attained its full magnitude, while the first response ceased to appear.

It can be decided without difficulty to which stimulus these responses are to be related if the latency of the responses is compared with that of

the normal unconditioned g.s.r. For instance, the first response must be related to the acoustic stimulus (M) because it appeared about 2 seconds after the beginning of M and the second response must have been caused by L because it began to rise about 2 seconds after the beginning of L.

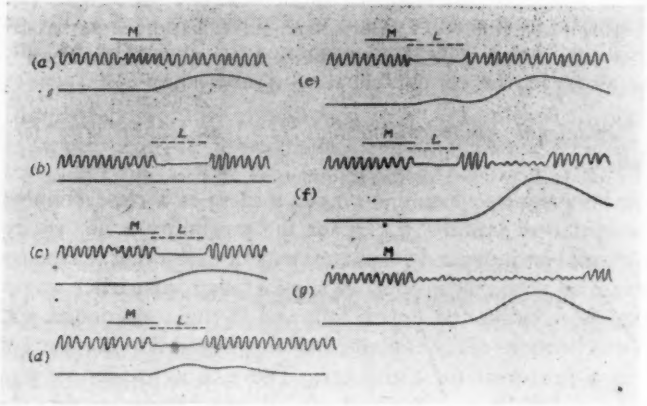


Fig. 1. Processes of conditioning. M: Metronome. L: Light. Explanation in the text.

The disappearance of the first response is an expression of complete conditioning, for in these states the conditioned stimulus alone evoked a remarkable g.s.r. which showed a latency of 4–6 seconds, as illustrated in (g) of Fig. 1. It is definitely longer than the latency of the normal g.s.r., and this very relation shows that this is a delayed conditioned reflex.

Now we must turn to the behaviour of the e.p. involved in the conditioned reflex. At the earlier stages of conditioning a brief e.p. appeared immediately after the onset of the acoustic stimulation (M). This sort of e.p., however, ceased to appear sooner or later and thereafter we had only the e.p. due to the light-stimulus (L). This sort of e.p. was always striking, but disappeared immediately after the cessation of the stimulation in the earlier half of the conditioning period. At later stages of conditioning a kind of after-potential was seen, which consisted of slight but long lasting excitation patterns. It must be noted that the after- potential is no direct continuation of the e.p. evoked by the light-stimulus, but that between them there exists a gap in which a burst of well developed α-waves is seen, as illustrated in (f) of Fig. 1. This curious feature can be explained by the assumption of so-called " temporal induction ", a physiological principle introduced by Sherrington to explain various phenomena in the central nervous system; The α-waves are strongly inhibited by the light-stimulus, and this inhibition induces the reversed

effect immediately after the termination of the stimulation to cause the observed temporary rise in α-activity.

The appearance of the after-potential is another expression of the complete conditioning, for it is very likely that the after-potential is a conditioned e.p. itself for the following reasons: 1. this kind of potential pattern appears only in later stages of conditioning, and 2. the pattern is hardly distinguishable from the conditioned e.p. evoked by the conditioned stimulus alone in the completely conditioned state.

2. *Conditioned Excitation-Potentials (e.p.) as the Precursor for External Reflex-Action.*

It is important to examine whether there is a close connection between conditioned e.p. and g.s.r.; for the possibility is not yet ruled out that they could be independent phenomena of each other. This possibility might even be probable in view of the fact that, at earlier stages of conditioning, the conditioned e.p. is followed by no conditioned g.s.r.; but this fact can be more easily explained by the assumption that the conditioned e.p. is a precursor for the g.s.r.: The e.p. at earlier stages of conditioning is generally so weak and of such short duration that it may be insufficient to set up impulses which could reach sweat glands of the skin to cause a g.s.r.

It depends solely upon the nature of e.p. whether it can cause any g.s.r. or not; the e.p. evoked by a simple light-stimulus is not followed by any strong g.s.r., how striking the depression of α-waves may be, because the nature of the potential pattern is quite different from that of the well established conditioned e.p., which is characterized by its inhomogeneous pattern and long duration. However if the light-stimulus happens to cause some mental processes such as unease, surprise etc., the pattern of e.p. is similar to that of the conditioned e.p. and it is followed by a g.s.r. As long as the cortical excitation is confined to the receptor mechanism as in the case of simple light-stimulation, the e.p. is simple and does not survive the stimulation, whereas an excitation strong enough to involve the effector mechanism gives rise to an e.p. chracterized by complexity and long duration as in the case of mental work. Because the conditioning is to be understood as the establishment of a functional association path between receptor- and effector mechanism in the cortex, it is quite natural that the conditioned e.p. should be very similar to that in mental work.

The study of brain-waves has revealed that there are many latent conditioned reflexes which can be detected at early stages of conditioning at which no external reflex-response yet appears. It seems that these latent conditioned reflexes have some influence upon our mental life, but

further studies are needed for elucidation of their psychological significance.

Another feature of our results which needs explanation, is that, at intermediate stages of conditioning, there appeared two g.s.r. of which the succeeding one increased in magnitude at the cost of the preceding one by further repetition of conditioning procedure. When the subject was excited experimentally by some extra-stimulus or spontaneously by some internal excitation in his completely conditioned state, there appeared two g.s.r. just like those at intermediate stages of conditioning. The extra-stimulus rendered the conditioned reflex less perfect so that double responses appeared. All these phenomena including the delay of conditioned e.p. and g.s.r. can be fully explained in terms of internal inhibition suggested by Pavlov. According to this author, the delay of the conditioned reflex is due to the action of internal inhibition which develops upon the conditioned stimulus and subsides in the lapse of a definite time depending upon experimental conditions, especially upon the interval between the beginning of the conditioned stimulus and the administration of the unconditioned stimulus.

There is a number of evidences that the internal inhibition can inhibit not only conditioned excitation in the cortex, but also all sorts of cortical excitation as well. Therefore, the unconditioned response upon the acoustic stimulus (M) cannot appear any more at advanced stages of conditioning, at which internal inhibition of sufficient strength develops, so that we have only the delayed conditioned response. If its development happens to be disturbed by any extra stimulus, it becomes too weak to inhibit the unconditioned excitation upon the acoustic stimulus (M) so that we have double g.s.r. The antagonistic relation between the conditioned and unconditioned responses can be explained very easily from this point of view.

3. *Generalization and Differentiation of Conditioned Reflexes.*

In order to make differentiation-experiments the sound from an electric bell was delivered to the subject in whom the delayed conditioned reflex had been established with the metronom (M) as the conditioned stimulus. The results are shown in Fig. 2. The record (a) of this Fig. represents the effect of the combined stimuli M and L, the broken lines over the electroencephalogram indicating the approximate duration of e.p. It must be noted that in this record only one g.s.r. appeared as the sign of complete conditioning and that between the two e.p. there appeared a very marked burst of α-waves due to temporal induction.

The effect of the conditioned stimulus alone is shown in the record (b) in the same Fig., in which a long lasting conditioned e.p. followed by a delayed g.s.r., is illustrated. The record (c) represents the result caused

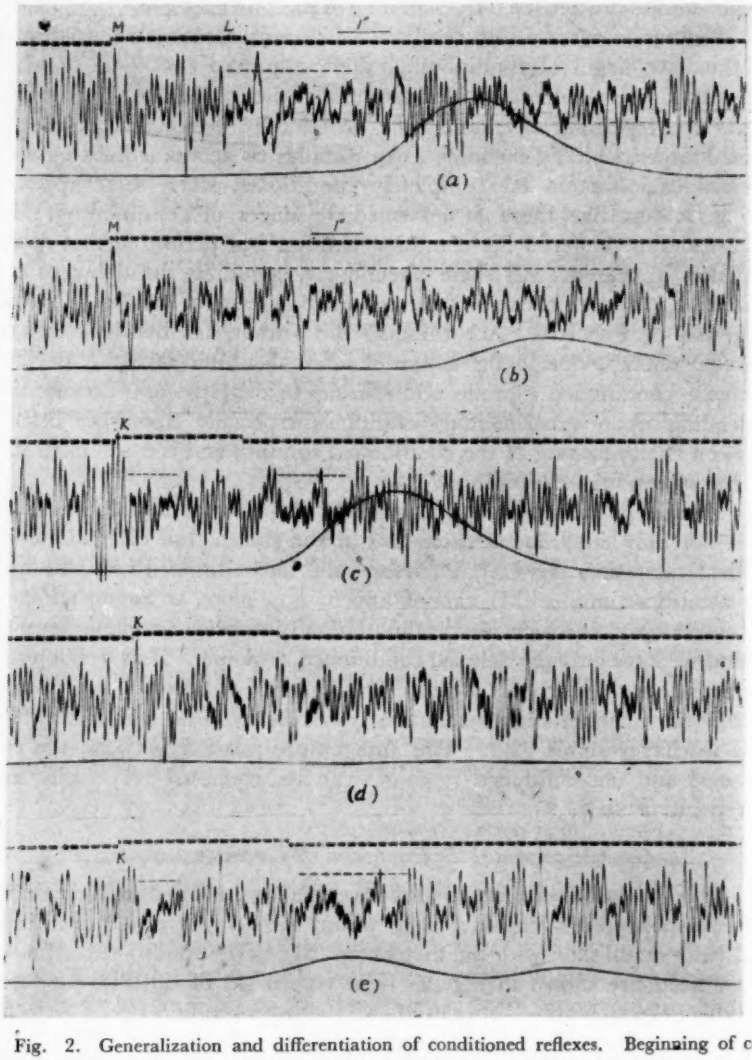


Fig. 2. Generalization and differentiation of conditioned reflexes. Beginning of conditioned and unconditioned stimulus marked with M and L. Broken lines indicate approximate duration of e.p.

(a): Effect of M+L after the establishment of conditioning. No unconditioned responses upon M. G.s.r. is related to L. Two e.p. are seen and between them a remarkable burst of α-waves (result of induction)

(b): Conditioned responses. e.p. has a complicated pattern and protracted course.

(c): Generalization upon the sound of a bell (K). Two e.p. but only one g.s.r. whose

latency is shorter than that of the conditioned g.s.r. in (b). The delayed e.p. is relatively short in duration.

(d): No changes upon K (differentiation).

(e): Generalization upon K in the mental excitement of the subject due to conversation with the investigator. Two e.p. and as many g.s.r. can be seen.

by the sound from the bell (K) which was given to the subject for the first time and without any previous notice. In this record two series of e.p. are seen; the preceding one which appeared immediately after the biginning of the stimulus (K) and lasted for about one second, is obviously the unconditioned e.p. evoked by K, but the succeeding one can be regarded neither as the direct effect of K nor as a conditioned e.p., because its latency and duration are too long to be accounted for as the unconditioned e.p. upon K and moreover this stimulus was not the conditioned stimulus for this experiment. In view of the fact that its latency is of the same order of magnitude as the delayed conditioned e.p. upon the conditioned stimulus (M), it must have appeared as the result of so-called generalization.

It is worth noticing that in spite of the double responses of the brain-waves there appeared only a single g.s.r., which, judging from its latency, must correspond to the preceding e.p. This means that the generalization of the conditioned reflex occurred only with respect to the e.p. In other words, the generalization in this case was of incomplete form: If the generalization had been perfect we should have had a delayed g.s.r. following the delayed e.p. From their experiments on dogs, Hayashi and his coworkers[4] concluded that no generalization takes place in delayed reflexes, and that this is one of the most conspicuous characteristics differentiating delayed conditioned reflexes from trace conditioned reflexes. This statement seems to hold good for the results of my experiments in man so long as the external response is concerned, but in view of the fact that the generalization took place with respect to the e.p. in the delayed conditioned reflex, it might be no fundamental difference between both sorts of conditioned reflexes whether the generalization takes place or not.

The appearance of the very marked unconditioned g.s.r. must partly be due to the circumstances that the internal inhibition initiated by the stimulus K was too weak to inhibit the unconditioned cortical excitation, the precursor for the unconditioned g.s.r., and partly due to the astonishment caused by the stimulus.

By repeated administrations of K the generalization was gradually reduced and finally the state was reached in which neither an e.p. nor a g.s.r. appeared, as illustrated in (d) of Fig. 2. In this state of the subject, however, the administration of the conditioned stimulus M always gave rise to a long lasting conditioned e.p. as well as a vigorous delayed g.s.r.,

thus somatic differentiation of both stimuli, M and K, having been accomplished. The example in Table I will serve to illustrate the time course of the establishment of differentiation, where the strength of e.p. and the magnitude of g.s.r. are expressed by the number of plus signs and galvanonometer deflections in mm. respectively. It may be noted that in the process of acquiring the differentiation capacity, there is a phase in which the conditioned reflex is considerablly reduced, e.g. the conditioned e.p. of +++ at the outset of the experiment was reduced to ++ and the g.s.r. was diminished by two thirds of its initial value.

As exemplified in this case and more conspicuously in the case of experimental extinction, conditioned g.s.r. are, in general, more easily reduced or extinguished than conditioned e.p., and this evidence supports the idea that the e.p. is a more essential feature of conditioning than the external response. The temporary reduction of conditioned reflexes in the process of differentiation is due to the development of internal inhibition which was brought about by the repetitive administrations of the stimulus K; as has been noted above, the internal inhibition not only inhibits the effect of the stimulus in question but also has a tendency to act against other sorts of excitation in the cortex, so that the conditioned excitation cannot be an exception and must fall a victim to it until the excitation is made by further conditioning strong enough to overcome the inhibition.

TABLE I.

Time course of the establishment of differentiation. Conditioned reflexes were established with a metronome (M) and a light (L) as conditioned and unconditioned stimulus respectively. The stimulus to be differentiated was the sound of a bell (K).

Time	Stimulus	Conditioned e.p.	Conditioned g.s.r. latency (sec.)	Conditioned g.s.r. strength (mm.)	Note
6 h 55′	M	+++	4.5	15	Cond. reflex before differentiation
59′	M+L	—	—	—	
7 h 5′	M+L	—	—	—	
8′	K	—	—	—	
12′	K	++	5.8	3	Generalization
18′	K	—	—	—	
22′	M	++	5.5	5	Reduction of cond. reflex
25′	K	+	5	2	Weak generalization
28′	K	0	—	0	Differentiation
32′	M+L	—	—	—	
35′	M+L	—	—	—	
38′	M	+++	4.5	22	Cond. reflex after differentiation

The differentiation capacity, even when well established and of long standing, is very likely to become greatly weakened or to disappear entirely at the intrusion of any extra stimulus (external inhibition). An example is illustrated in record (e) of Fig. 2. The extra stimulus in this case consisted of the conversation between subject and experimenter who inquired of the subject about his psychical state during the experiment. Immediately hereafter the stimulus K was delivered, in order to see whether his differentiation capacity remained unaltered, and it was found that K was no more so indifferent as before, but able to evoke unconditioned as well as delayed responses, though slight, as indicated in (e) of Fig. 2. The most interesting aspect of this record is that two galvanic skin-responses are seen, what was, however, not the case in the record (c).

At any rate, the way of coupling between e.p. and g.s.r. realized in our experiments may be classified into the following types:

1. Type of perfect conditioning. Conditioned e.p. conditioned g.s.r.
2. Type of imperfect conditioning.
   - A. Uncond. e.p. uncond. g.s.r.
     cond. e.p. cond. g.s.r.
   - B. Uncond. e.p. uncond. g.s.r.
     cond. e.p.
3. Type of non conditioning.
   - A. Uncond. e.p. uncond. g.s.r.
   - B. Uncond. e.p.

As clearly seen in this table, all g.s.r. are preceded by e.p. but e.p. are not always followed by g.s.r., indicating that e.p. furnishes the finest and most reliable means for the study of conditioned reflexes.

Summary

1. In order to establish conditioned responses of brain-waves, a weak acoustic stimulus M which was almost ineffective for evoking any excitation-potential (e.p.) was applied many times with an interval of about 3–5 minutes associated with a light which was delivered 2 seconds after the beginning of the stimulus M. So obtained e.p. was characterized by complicated patterns and long duration and very similar to the e.p. caused by mental work.

2. Though the light-stimulus used as the unconditioned stimulus was in general little effective for evoking galvanic skin-responses (g.s.r.), a remarkable g.s.r. accompanied the e.p. caused by the conditioned stimulus. This fact indicates that the conditioned e.p. was no more confined to the receptor mechanism in the cortex, but involved the effector mechanism.

3. When another sort of acoustic stimulus K was applied in the completely conditioned state of the subject, the generalization of the

conditioned reflex took place in such a manner that a delayed e.p. appeared alone or sometimes followed by a g.s.r.

4. By further repetitions of K a state was reached in which conditioned responses appeared only upon M but not upon K (differentiation).

In all these phenomena, e.p. are easier to be conditioned and more difficult to be extinguished than external responses. It may be concluded that e.p. are precursors for external responses and faithful indicators of the central mechanism of conditioning.

References

(1) Motokawa, K. & B. Huzimori, Tohoku J. exp. Med., 1949, **50.**
(2) Motokawa, K. & B. Huzimori, Jap. J. Med. Sci., Biophys., 1945, **10**, 366.
(3) Loomis, L., E. Harvey & G. Hobart, J. exp. Psychol., 1936, **19**, 249.
(4) Hayashi, T., Dainoserigaku, 1944, Tokyo, p. 385.

The Tohoku Journal of Experimental Medicine, Vol. 50, Nos. 3 & 4, 1949.

The Salt Error of Brom-thymol-blue.

A Note on the Colorimetric Determination of pH with Brom-thymol-blue at 38° C.

By

Kojiro Matsuda and Yoshishichiro Koyama.

(松田 幸次郎) (小山 義七郎)

(From the Department of Aviation Medicine, Faculty of Medicine, Tohoku University, Sendai.)

(Received for publication, April 18, 1945)

Brom-thymol-blue (B.T.B.) is one of the acid-base indicators which are most frequently used in biological works becuase its range of color change lies at the neutral region of reaction. Generally speaking the colorimetric method of pH determination with acid-base indicators requires no particular and complicated appratus and is preferred in simplicity and rapidity to the more accurate but laborious electrometric methods, but is liable to error if it be undertaken without proper precautions. The fact that the colors of a certain indicator in solutions which are the same in pH but are different in concentrations of electrolytes are not the same is called " salt error " and should always be taken into consideration in the colorimetric pH determination. The cause and extent of this salt error was discussed by several authors (Clark[1], Kolthoff[2], Sendroy and Hastings[3]) but the data published up to date are not yet complete. For example as we tried to use B.T.B. as the indicator for the colorimetric determination of pH of human saliva at body temperature we could find no data dealing with the salt error of B.T.B. at 38°C. Therefore from the practical need we had to determine the actual value of the salt error by experiments, the result of which we would present in this report.

To interprete the color change of B.T.B. according to the reaction, this dye may be regarded as a dibasic acid, its mono and divalent anions giving yellow and blue color respectively. The equilibrium can be expressed as

$$pH = pK_2 + \log \frac{a_{A''}}{a_{HA'}}.$$

pK_2: The second ionization exponent of B.T.B.

$a_{A''}$, $a_{HA'}$: The activity of monovalent and divalent anions respectively.

The actual values of activities being not known, the above equation may be transformed as:

$$pH=pK_2+\log\frac{\gamma_{A''}[A'']}{\gamma_{HA'}[HA']}. \qquad (1)$$

If we put $pK_2+\log\frac{\gamma_{A''}}{\gamma_{HA'}}=pK'_2$, (2)

equation (1) will be

$$pH=pK'_2+\log\frac{[A'']}{[HA']}. \qquad (3)$$

Here $\gamma_{A''}$, $\gamma_{HA'}$: Activity coefficient of indicator ions respectively.
pK'_2: Apparent second ionization exponent of B.T.B.
[A''], [HA']: Stoichiometric concentration of indicator inos respectively.

If we know log [A'']/[HA'] (usually designated as log R) of the indicator in the sample solution by comparison with proper color standards the pH of the solution may be obtained by equation (3) provided pK'_2 be also known. But as pK'_2 usually changes as the nature and concentration (or more adequately ionic strength) of electrolytes in the solution changes, pH value calculated by equation (3) assuming pK'_2 to be constant throughout, will be erroneous. Thus the "salt error" occurs. To eliminate the error we must know previously the value of pK'_2 for each solution and for each temperature in consideration. Such pK'_2 values can be obtained from (3) if one measures the true pH of solutions of various compositions with the hydrogen electrode and at the same time reads the value of log R of B.T.B. in these solutions by means of colorimetry.

Experiment and its result.

The solutions investigated are phosphate buffer mixtures and sodium chloride solutions of varying concentrations. These were prepared by dilution of stock solutions: (1) 1/16 M phosphate buffer mixture, $Na_2HPO_4:KH_2PO_4=1:1$ and (2) 1.45 M sodium chloride solution. Reagents used were all Kahlbaum's preparations "pro analysi." In cases of sodium chloride solutions phosphate buffer was added to a small extent to stabilize their pH. This time, solutions of electrolytes other than above were not studied.

All measurements by means of the hydrogen electrode were carried out in an liquid thermostat of 38°±0.05°C. The electrode used was of the bubbling hydrogen type and was combined with the saturated calomel half cell by means of saturated KCl bridge. As the standard, pH value of 0.01 N HCl was adopted to be 2.036. This standard yields almost the same results as the assumption that pH of 0.1 N HCI is 1.08 at 38°C. The comparison of indicator colors in solution was performed according

to Hastings and Sendroy using the bicolor standards prepared exactly as their direction except that the concentration of B.T.B. in the solutions to be tested was made 0.002%. The dye B.T.B. used was a Japanese preparation of Takeda Chemical Manufacturing Co.; we often compared this preparation and Merck's B.T.B. but in any case no difference in results was noticed. Comparison of colors was performed always at 38°C, i.e. solutions to be examined was warmed previously in a water bath.

The results are summarized in table 1 and 2.

TABLE 1.

Electrometric and colorimetric pH of phosphate buffer mixtures of various ionic strength at 38°C.

Concentration of phosphate (Mol/l)	Ionic strength μ (Mol/l)	$\sqrt{\mu}$	Electrometric pH	log R	pK'_2
0.0667	0.133	0.365	6.797	−0.29	7.09
0.0445	0.0890	0.298	6.841	−0.26	7.10
0.0333	0.0666	0.258	6.873	−0.24	7.11
0.0167	0.0333	0.183	6.944	−0.21	7.15
0.00834	0.0167	0.129	7.000	−0.20	7.20
0.00416	0.00834	0.0908	7.030	−0.19	7.22
0.00267	0.00533	0.0725	7.039	−0.19	7.23
0.00208	0.00416	0.0645	7.046	−0.26	7.31

TABLE 2.

Electrometric and colorimetric pH of NaCl solutions of various ionic strength at 38°C.

Concentration of NaCl (Mol/l)	Total ionic strength μ* (Mol/l)	$\sqrt{\mu}$	Electrometric pH	log R	pK'_2
0.308	0.313	0.559	6.515	−0.41	6.93
0.231	0.236	0.486	6.574	−0.39	6.96
0.154	0.159	0.399	6.630	−0.38	7.01
0.0770	0.0823	0.287	6.744	−0.33	7.07
0.0308	0.0361	0.190	6.856	−0.29	7.15
0.0154	0.0207	0.144	6.918	−0.27	7.19
0.00770	0.0130	0.114	6.940	−0.26	7.20

* Phosphate mixture (μ=0.00533) was added to stabilize pH.

Discussion.

In Fig. 1 the experimentally obtained pK'_2 are plotted against the ionic strength (μ) and its square root ($\sqrt{\mu}$). Plotting against $\sqrt{\mu}$ is to make possible to extrapolate the curve to $\mu \to 0$ in order to get pK_2, the second thermodynamic ionization exponent of B.T.B. at 38°C. According to the Debye-Hückel's theory, in sufficiently dilute solutions $pK'_2 - pK_2$ i.e. log $(\gamma_{A''}/\gamma_{HA'})$ may be expressed as

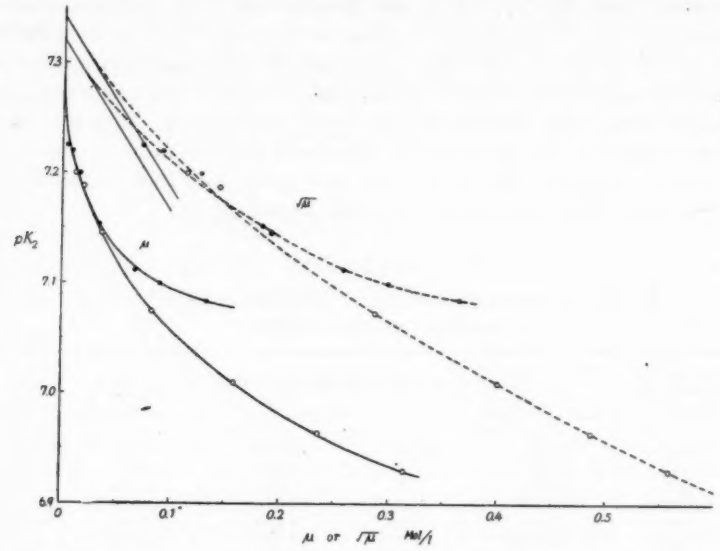


Fig. 1. Relation between pK'_2 of B.T.B. and ionic strength (μ) of phosphate mixture (•) or of NaCl solution (○).
Solid line: $pK'_2 \sim \mu$ curve,
Broken line: $pK'_2 \sim \sqrt{\mu}$ curve.

$$\log \frac{\gamma_{A''}}{\gamma_{HA'}} = -\frac{3A\sqrt{\mu}}{1+\beta a\sqrt{\mu}}. \qquad (4)$$

Here A: 0.5184 } both are constants at 38° C in Debye-Hückel's
β: 0.3314×10^6 } equation.[4]
a: Average ionic diameter in the solution.

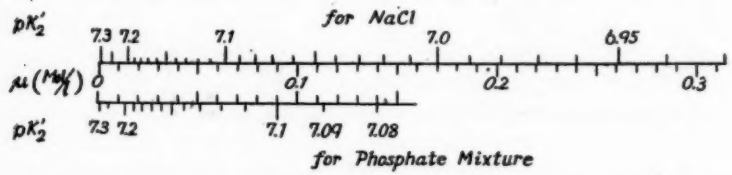


Fig. 2. Functional scale to read pK'_2 of B.T.B. at 38°C at any ionic strength (μ) of phosphate mixture or of NaCl solution.

In infinite dilution the right side of the above equation becomes $-3A\sqrt{\mu}$. Therefore the experimental curve of pK'_2 plotted against $\sqrt{\mu}$ is to be extrapolated to $\mu\rightarrow0$ so as to cut the ordinate axis as a straight line with direction coefficient of $-3A$. The value of ordinate at the point of ex-

trapolation to $\sqrt{\mu} \rightarrow 0$ is pK_2. By such a procedure $pK_2 = 7.32$ for phosphate mixtures, 7.34 for sodium chloride solutions were obtained. From theory both values should agree. The small difference is probably due to experimental errors (e.g. neglected diffusion potentials etc.). The average is 7.33 at 38°C. pK'_2 values for each μ of phosphate and sodium cholride solutions are represented in a scale form in Fig 2. Using these values of pK'_2 of B.T.B. we can get true pH without salt errors at 38° C from equation (3). Even when the compositions of sample solutions are not exactly known, pK'_2 for approximate or equivalent concentration of sodium chloride will be used without gross errors for biological materials.

In the above discussion pK'_2 is assumed to be influenced solely by the ionic strength except the temperature and the nature of electrolyte, but this is no more tenable in general. Some corrections due to log R may be taken into consideration but in so far as μ remains to be fairly low such as in our present report this effect may be practically negligible (Cohn[5]).

Summary.

The apparent ionization exponent pK'_2 of B.T.B. at 38°C was determined in phosphate mixtures and solutions of sodium chloride of various ionic strength up to 0.133 Mol/l in phosphate mixtures and 0.308 Mol/l in sodium chloride solutions.

The thermodynamic ionization exponent pK_2 obtained by extrapolation to $\mu \rightarrow 0$ is 7.33 at 38°C.

pK'_2 values thus obtained make possible to eliminate the " salt error " of B.T.B. and can be profitably used for pH determination of biological fluids at body temperature.

References.

(1) Clark, M., The Determination of Hydrogen Ions, 1928, Baltimore.
(2) Kolthoff, I.M., Acid-base Indicators, 1937, New York.
(3) Sendroy, J. and B. Hastings, Journ. Biol. Chem., 1929, **82**, 197.
(4) MacInnes, D.A., The Principles of Electrochemistry, 1938, New York. p. 144.
(5) Cohn, E.J., Journ. Amer. Chem. Soc., 1927, **49**, 173.

Color Receptors in Frog Eyes as Revealed by Electrical Stimulation.

By

Kōiti Motokawa and **Kituya Iwama.**

(本川 弘一)　　(岩間 吉也)

(From the Physiological Laboratory of Prof. K. Motokawa, Tohoku University, Sendai.)

(Received for publication, April 20, 1949)

We have found that the electrical excitability of excised frog eyes as determined with action potentials of the optic nerve as the index of excitation, shows characteristic variations after a brief illumination of the eye by various colored lights.

The stimulating constant current was applied to the eye through a pair of non-polarizable electrodes of Zn-$ZnSO_4$-gelatine type, of which one was placed on the cornea and the other on the posterior pole of the eye ball. The former electrode was connected to the negative pole of the current source. The duration of the current was one second. The illumination was two seconds in duration and restricted to the area centralis. The action potentials were observed on the screen of a cathode ray oscillograph.

The data are illustrated in Fig. 1, in which the percentage increase in electrical excitability is plotted against the time after the end of illumination. The excitability curve shows generally three humps, but sometimes only two, as seen in the figure, when white light is used for pre-illumination. The time course of the excitability curve is the shortest, intermediate and the longest, when red (740–620 mμ), green (550–490 mμ) and blue (490–410 mμ) lights are used for pre-illumination respectively. The excitability curve for white light represents the envelope of the curves obtained for the three colored lights mentioned above.

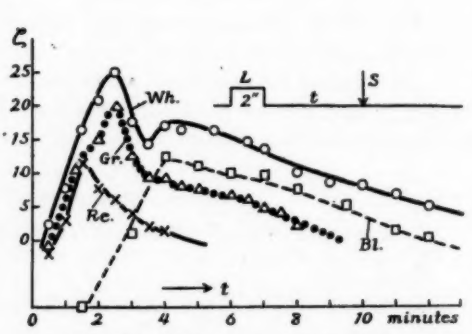


Fig. 1. Excitability curves of the frog eye for white (Wh.), red (Re.), green (Gr.) and blue (Bl.) light respectively. L: light. S: electrical stimulation. The curve Wh. is drawn displaced upwards by 5 units of ζ.

The Tohoku Journal of Experimental Medicine, Vol. 50, Nos. 3 & 4, 1949.

Über tuberkulöse Bazillämie bei Kaninchen, die mit BCG vorbehandelt wurden.

Von

Hideo Maeta.

(前田 秀雄)

(Aus der Medizinischen Klinik und dem Kosankinbyo Kenkyusyo der Kaiserlichen Tohoku-Universität zu Sendai. Leiter: Prof. Dr. T. Kumagai und Prof. Dr. T. Ebina)

(Received for publication, June 21, 1945)

Einleitung.

Über die immunisatorische und prophylaktische Wirkung der BCG, insbesondere im Tierversuch liegen schon zahlreiche Arbeiten vor. Aber über die hemmende Wirkung der BCG-Impfung auf die Entwicklung der Bazillämie, die durch nachträglich intravenös eingesprizte Tuberkelbazillen verursacht wird, sind bis jetzt nur wenige Untersuchungsergebnisse veröffentlicht worden.

Wir haben uns mit dieser Frage beschäftigt und unsere Beobachtungen seien im folgenden mitgeteilt.

Methodisches.

Als Versuchstiere wurden gesunde Kaninchen von ca 2 Kg gewählt, welche auf intrakutan eingespritztes Tuberkulin negativ reagierten. Verwendet wurden BCG, die auf dem Lockemannschen Nährboden gut wuchsen.

Um die experimentelle Bazillämie hervorzurufen verwendete man virulente Tuberkelbazillen eines humanen Stammes (Stamm Sato) unseres Laboratoriums. Zur Blutkultur entnahm man 2,0 ccm Venenblut. Dieses wurde in einem Spitzglas mit 18 ccm einer 0,1% Saponin und 0,25% Calciumhydrochlorid enthaltenden Lösung vermischt, vorsichtig geschüttelt, dann 12 Stunden lang bei Zimmertemperatur gehalten. Danach wurde das Glas 10 Minuten lang mit 3000 Umdrehungen zentrifugiert, die dabei entstehende obere klare Schicht abgenommen, wiederum 18 ccm destilliertes Wasser hinzugefügt, sanft geschüttelt und abermals zentrifugiert. Dem Niederschlag wurden unter sanftem Schütteln des Glases 5 Tropfen einer 4% igen Schwefelsäure, die in 100 ccm 5,0 gr

granuliertes Pepsin enthielt, hinzugefügt. Nach 30 Minuten wurde dieser weich gemachte Niederschlag auf drei Eiernährböden nach Oka- Katakura[1]) eingerieben, dann im Brutschrank aufbewahrt. 2 Monate danach wurden die Kolonien auf dem Nährboden mit blossen Augen gezählt.

Untersuchungsmethode.

Die zu verwendenden gesunden Kaninchen wurden in zwei Gruppen eingeteilt. Die erste Gruppe bildeten 13 Kaninchen, unter denen bei 4 Fällen 1,0 mg, bei 3 Fällen 5,0 mg, bei 4 Fällen 10,0 mg und bei 1 Fall 20,0 mg lebender BCG intrakutan eingeimpft wurden. Die zweite Gruppe bildeten 16 andere nichtvorbehandelte Kaninchen. Den Tieren beider Gruppen wurden 23,48 oder 82 Tage nach der BCG-Impfung je 1,0 mg virulente Tuberkelbazillen intravenös injiziert.

Im Anschluss daran wurden in Abständen von 1 bis 7 Tagen Blutproben entnommen und nach obengenannter Methode 13 mal Blutkulturversuche durchgeführt.

Dauer der Bazillämie und Zahl der Tbk-Bazillenkolonien.

Von 12 bis zum Ende des Versuchs nicht gestorbenen Kaninchen der ersten, mit BCG vorbehandelten Gruppe wurden bei 2 Fällen (Nr. 5 und 14) nur einen Tag nach der Infektion positive Kulturen erhalten und die Zahl der beobachteten Kolonien betrug 15—O und 1—O: bei 4 Fällen (Nr. 54, 53, 52 und 19) waren die Kulturen 3 Tage lang positiv und wurden 8–3, 14–1, 21–4 und 60–10 Kolonien gezählt: bei 2 Fällen (Nr. 1 und Nr. 4) waren die Kulturen 7 Tage lang positiv und betrugen die Kolonienzahlen 20–1 und 300–2: 1 Fall 10 Tage positiv, Kolonienzahl 14–1: 1 Fall (Nr. 32) 14 Tage lang positiv, Kolonienzahl 34–1: 1 Fall (Nr. 56) 20 Tage lang positiv Kolonienzahl 30–20: 1 Fall (Nr. 55) 25 Tage lang positiv, Kolonienzahl 15–1. Bei 8 bis zum Ende des Versuchs nicht gestorbenen Kaninchen der zweiten, nicht vorbehandelten Gruppe wurde bei einem Fall (Nr. 70) während 20 Tagen nach der Infektion positive Kultur nachgewiesen, die dabei entstandenen Kolonien betrugen 120–3: 1 Fall (Nr. 67) war 25 Tage lang positiv, die Kolonien Zahl betrug 140–1: 4 Fälle (Nr. 68, 64, 63 und 40) 35 Tage lang positiv, Kolonien 116–1, 120–7, 150–1: 2 Fälle (Nr. 29 und 65) 62 Tage positiv, Kolonien 42–1 und 270–1. Bei dem am 41. Tag nach der Infektion gestorbenen Fall der ersten, vorbehandelten Gruppe liessen sich die Tuberkelbazillen während 35 Tagen nach der Infektion kulturell nachweisen, die Zahl der dabei entstandenen Kolonien betrug 17–1. 8 Fälle der zweiten, nicht vorbehandelten Gruppe starben. Bei 5 innerhalb 25 Tagen nach der Infektion gestorbenen Fällen (Nr. 42, 23, 21, 43 und 41) wurde bis zum Tod stetz positive Züchtung erzielt, dabei treten die Kolonien meist zahlreich auf. Die anderen 3 Tiere starben 35–55 Tage nach der

Infektion. Bei ihnen konnten wir relativ wenige Bazillen züchten.

Aus der Tabelle I kann man ersehen, dass aus dem Blut der mit BCG vorbehandelten Kaninchen Kulturen virulenter Tuberkelbazillen, die nachträglich intravenös eingeführt worden waren, weniger und kürzere Zeit erhalten werden konnten als aus dem Blut der nicht mit BCG vorbehandelten Tiere.

Kurz gesagt, bei den vorbehandelten Fällen konnte man Hemmung der Bazillämie, die durch nachträglich injizierte virulente Tuberkelbazillen verursacht war, infolge BCG-Impfung feststellen.

Allergie und Bazillämie.

Bei den nicht vorbehandelten Tieren wurden ausserdem die Zusammenhänge der von den injizierten Tbk-Bazillen hervorgerufenen Tuberkulin-Allergie mit der Bazillämie untersucht. Wie die Tabelle zeigt, war die Dauer der Bazillämie desto kürzer, je früher die Tuberkulinreaktion positiv ausfiel. Bei den 6 bis zum Tod nicht auf Tuberkulin reagierenden Fällen konnte man bis zum Tod Bazillämie nachweisen. Einen direkten Zusammenhang der Tuberkulinreaktionsstärke mit der Bazillämie-Dauer konnten wir aber nicht finden.

Ferner wurde das Verhältnis der durch die BCG-Impfung hervorgerufenen Tuberkulin-Allergie zu der durch die sekundär injizierten virulenten Tuberkelbazillen verursachten Bazillämie untersucht. Wie die Tabelle zeigt, konnten wir eine enge Beziehung zwischen diesen beiden Erscheinungen feststellen: die Kaninchen, bei denen die durch die BCG-Impfung erzeugte Tuberkelallergie später auftrat, zeigten eine länger andauernde durch die virulenten Tuberkelbazillen hervorgerufene Bazillämie als die, bei denen die Tuberkulinreaktion frühzeitiger positiv ausfiel.

Einen wichtigen Zusammenhang zwischen der zur Impfung angewendeten BCG-Menge und der durch die virulenten Tbk-Bazillen erzeugten Bazillämie konnte wir in unseren Versuchen nicht feststellen.

Bazillämie und Sektionsbefunde.

Eine interessante Frage ist auch die, ob die Tuberkelbazillämie anderen pathologischen Veränderungen parallel geht. Alle Tiere, die während des Versuches starben, wurden gleich nach dem Tod seziert und die am Leben gebiebenen 63 Tage nach der Infektion mit virulenten Bazillen zwecks Feststellung der pathologischen Veränderungen getötet. In der Tabelle I habe ich die bei verschiedenen Organen beobachteten tuberkulösen Veränderungen und die Dauer der tuberkulösen Bazillämie zusammengestellt.

Bei den mit BCG-vorbehandelten Tieren ist keine enge Beziehung zwischen der Zeitdauer, in der man die Tuberkelbazillen im Kulturversuch nachweisen kann, und den pathologischen Veränderungen zu finden.

Tabelle I.

Nr. Kaninchen	Menge der BCG (mg)	Tage bis Positiverwerden der Tuberkulinreaktion durch BCG	Tuberkulinreaktion gleich vor Tbk.-Baz.-Infektion	Menge der virulenten Tbk.-Baz. (mg)	Tage bis Positivwerden der Tuberkulin reaktion durch virulenten Tbk.-Baz.	Tage nach der Infektion, wo Bazillämie nachgewiesen												Positive Kultur-dauer	Tage bis Tod nach Infektion mit Tbk.-Baz.	Sektionsbefund					
						1	3	7	10	14	20	25	30	35	42	47	55	62		Lunge	Milz	Leber	Niere	Darm	Lymph-drüse
5	10,0	13	15×15	2,0		15	—	—	—	—	—	—	—	—	—	—	—	—	1	±					
54	5,0	9	70×70	1,0		8	3	—	—	—	—	—	—	—	—	—	—	—	3	±		±	—		
53	5,0	12	40×40	1,0		10	60	—	—	—	—	—	—	—	—	—	—	—	3	±		±	±		
1	1,0	13	25×25	2,0		300	1	2	—	—	—	—	—	—	—	—	—	—	7	±		±	±	+	
4	5,0	13	20×20	2,0		7	29	1	—	—	—	—	—	—	—	—	—	—	7	±		±	±	±	
2	1,0	22	25×25	1,0		—	5	1	14	—	—	—	—	—	—	—	—	—	10	±		±	+	±	
14	5,0	15	25×25	1,0		1	—	—	—	—	—	—	—	17	—	—	—	3	1	±		±	±	±	
52	1,0	12	25×20	1,0		4	21	—	—	—	—	—	—	—	—	—	8	—	3	55	62	±				
13	10,0	15	15×15	1,0		9	1	—	—	—	—	4	—	14	—	1	—	1	9	35	62	±		+		
32	1,0	18	30×30	1,0		32	1	34	7	—	—	—	—	—	—	1	10	1	62			±				
56	10,0	19	35×35	1,0		20	30	—	30	—	20	—	—	1	—	—	—	—	35			±				
55	10,0	10	30×30	1,0		15	10	—	5	—	10	10	—	—	—	10	—	—	25	47		±				
11	20,0	21	15×15	1,0		7	—	10	1	7	10	1	—	(+)	—	—	—	—	35	+		±				
70				1,0	9	16	120	20	3	—	18	—	—	—	—	—	—	—	20			+				
67				1,0	9	2	65	1	140	—	40	52	—	—	—	—	—	—	62			±				
68				1,0	22	—	116	—	94	45	1	—	1	15	—	—	—	—	25			±				
64				1,0	22	120	56	7	30	—	120	—	—	10	—	—	—	—	35			±				
63				1,0	22	3	150	50	18	—	110	1	1	108	—	—	—	—	35			±				
40				1,0	19	150	128	80	1	10	17	1	—	123	—	—	—	—	35			±				
29				1,0	30	1	—	8	—	42	—	2	—	19	6	1	—	1	62			±				
65				1,0	22	75	270	130	84	51	250	23	1	34	30	211	—	73	62			±				
22				1,0	—	3	87	3	—	—	2	80	—	—	21	9	(+)		47	+						
27				1,0	30	11	1	12	5	—	2	—	—	49	—	1	(+)		47	+						
28				1,0	—	3	—	62	—	10	63	34	1	(+)					20	+						
42				1,0	16	103	47	113	49	13	1	(+)							25	+						
23				1,0	—	120	4	7	22	1	(+)								14	+						
21				2,0	—	11	47	43	12	(+)									19	+						
43				1,0	—	52	27	17	170	(+)									13	+						
41				1,0	—	61	40	100	65	(+)									12	+						
																			11	+						

(+) . . . gestorben Nr. 1, 4, 5, mit 2 mg Tuberkelbazillen infiziert.

Bei den nichtvorbehandelten Tieren, die während des Versuches starben, sind makroskopisch keine deutlichen pathologischen Veränderungen nachweisbar, obgleich bei ihnen die Bazillämie immer positiv war; bei den ohne BCG-Impfung getöteten Kaninchen aber trat die tuberkulöse Bazillämie länger auf und waren die pathologischen Veränderungen deutlicher, als bei den mit BCG vorbehandelten.

Nach Corper u. Vidal,[2] Tiedemann,[3] Strempell.[4] Mischlow u. Park,[5] Guichard,[6] Duchaine,[7] Ueda[8] und Irimami[9] soll bei den von 1,0–0,1 mg virulenten humanen bzw. bovinen Tbk-Bazillen intravenös infizierten Kaninchen ca 2 Wochen lang positive Kultur erhalten werden. Auch bei den von mir beobachteten Kaninchen konnte ich in einem Zeitraum von 20 bis 62 Tagen positive Kulturen erzielen, obgleich ihre zeitliche Schwankung sehr gross und unregelmässig war.

Im Bezug auf den Einfluss der BCG-Impfung auf die Bazillämie, die sekundär durch virulente Tuberkelbazillen verursacht wurde, beobachteten Ninni et Monaldi[10] und Duchaine[11] eine deutlich hemmende Wirkung. Wir haben in unseren Versuchen gesehen, dass bei mit BCG vorbehandelten Tieren 1–10 Tage nach der Tuberkelbazilleninfektion relativ wenig Kolonien aus Blut gezüchtet werden konnten. Bei den nichtvorbehandelten Tieren, deren Tuberkulinreaktion innerhalb kurzer Zeit positiv wurde, währte die tuberkulöse Bazillämie 20–25 Tage, während aus dem Blut der Tiere ohne BCG-Impfung, bei denen die Tuberkulinreaktion noch später positiv ausfiel, 35–62 Tage lang Bazillen kulturell nachweisbar waren.

In unseren Versuchen konnten wir auch die Schutzwirkung der BCG gegenüber der Tuberkuloseinfektion nicht nur in der hemmenden Wirkung auf die Bazillämie, die sekundär durch virulente Tuberkelbazillen verursacht wird, sondern in der Sterblichkeit durch virulente Tuberkelbazillen kennen lernen.

Ferner gab Bacanu[12] an, dass bei den mit BCG vorbehandelten und nachträglich infizierten Tieren eine Verspätung im Auftreten der Bazillen in den Organen festzustellen war. Andererseits sprachen Bouquet et Laporte[13] die Ansicht aus, dass während der ersten 2 Wochen nach der Superinfektion die Bazillen an Ort und Stelle liegen bleiben und erst später in Lymphknoten gefunden werden. Wir haben auch beobachtet, dass die Lungen der mit BCG vorbehandelten und nicht vorbehandelten Kaninchen fast gleich stark, aber andere Organe der letzteren in der Mehrzahl der Fälle stärker befallen waren, als bei den ersteren. Dies bedeutet, dass die Erkrankung sich bei den nichtvorbehandelten auf dem Blutwege schneller allgemein verbreitet als bei den mit BCG vorbehandelten.

Aus den oben besprochenen Beobachtungen an meinen Versuchstie-

ren darf man folgende Schlüsse ziehen.

1) Bei zuerst mit BCG vorbehandelten und dann mit virulenten Tuberkelbazillen intravenös infizierten Kaninchen ergeben Kulturversuche nur kürzere Zeit und in geringerer Anzahl Tuberkelbazillen als bei den nicht vorbehandelten.

2) Aus dem Blut der Kaninchen, bei denen virulente Tuberkelbazillen intravenös injiziert werden, lassen sich Tuberkelbazillen aus dem Blut desto leichter kultivieren, je früher der Kulturversuch nach der Infektion angestellt wird, sehr schwer aber in späteren Stadien.

Diese Forschung wurde durch überlassung von Geldmitteln des Unterrichtsministeriums für wissenschaftliche Forschung ausgeführt, wofür herzlich gedankt sei. Prof. Dr. T. Kumagai und
Prof. Dr. T. Ebina.

Literatur.

(1) Oka-Katakura, Tohoku Ig. Z., 1939, **24,** 71. Nihon Rinsyo Kekkaku, 1940, **1,** 829.
(2) Corper u. Vidal, Amer. Rev. Tbc., 1935, **32**, 575.
(3) Tiedemann, Beitr. Klin. Tbk., 1932, **81**, 450.
(4) Strempel, Zbl. Bak. I orig., 1934–35, **133**, 159.
(5) Mischlow u. Park, J. Prev. Med., 1932 **28**, 875.
(6) Guichard, Beitr. Klin. Tbk., 1932, **80**, 285.
(7) Duchaine, Amer. Rev. Tbc., 1938, **37**, 507.
(8) Ueda, Nihon Biseibutu Z., 1939, **33,** 1465; 1940, **34**, 45.
(9) Irimami, Hukuoka acta Med., 1942, **35**, 1064.
(10) Ninni et Monaldi, C.r. Soc. Biol., Paris, 1933, **113**, 377.
(11) Duchaine, Amer. Rev. Tac., 1938, **37**, 520.
(12) Bacanu, C. r. Soc. Biol. Paris, 1935, **120,** 1061.
(13) Bouqet et Laporte, Zbl. Tbk-Forsch., 1936, **44**, 501.

The Tohoku Journal of Experimental Medicine, Vol. 50, Nos. 3 & 4, 1949.

Ueber die erreichbare Genauigkeit der Kulturmethode zum Nachweis von Tuberkelbazillen.

Von

Risyun Yasumoto.
(安本 利俊)
(Aus der Medizinischen Klinik und dem Kosankinbyo Kenkyusyo der Kaiserlichen Tohoku-Universität zu Sendai. Letier: Prof. Dr. T. Kumagai und und Prof. Dr. T. Ebina.)

(Received for publication, March 23, 1946)

I. Einleitung

Es versteht sich von selbst, welch grosse klinische und sozilahygienische Bedeutung der Nachweis von Erregern der Infektionskrankheiten hat. Die Tuberkulose als eine chronische Infektionskrankheit macht keine Ausnahme. Darum ist es selbstverständlich, dass sich bisher viele Autoren sehr um den Nachweis der Tuberkelbazillen bemüht haben. Bei uns in Japan waren die Bemühungen Prof. Kumagais[1)2)] und seiner Schüler um die Kultur dieser Bakterien klinisch und technisch von Erfolg gekrönt, so dass jetzt diese ursprünglich nur schwer zu kultivierenden Krankheitserreger überall kultiviert werden können.

Darüber, in welcher Menge Tuberkelbazillen im zu untersuchenden Material vorhanden sein müssen, um durch Kultur nachgewiesen werden zu können, wissen wir indessen bis jetzt nur sehr wenig. Butschowitz[3)] gelang es, aus Sputum mit 1 mg Bakterienmasse pro 1 ccm—nach ihm enthält 1 mg Bazillenmasse 37,000,000 Tuberkelbazillen—29% lebende durch Kultur herauszuzüchten. Iibuti[4)] teilte mit, dass man, wenn Tuberkelbazillen in das Blut eingeführt werden, bei der Mischung von $\frac{1}{1{,}000{,}000}$ mg Bakterienmasse pro 1 ccm eine, bei einer solchen von $\frac{1}{500{,}000}$ mg 2 und bei der von $\frac{1}{100{,}000}$ mg 15 Kolonien herauszüchten kann. Katakura, Oka[5)] u.a. konnten aus $\frac{1}{500{,}000}$ mg Bazillenmasse 5, aus $\frac{1}{2{,}500{,}000}$ mg 2 und aus $\frac{1}{5{,}000{,}000}$ mg eine Kolonie nachweisen. Okada[6)] unter-

suchte bei Lungentuberkulösen den Bakterienbefund in der Sputum-Tagesmenge gleichzeitig durch Ausstrichpräparate und Kultur und konnte 10% Bazillen aus Aputum von Gaffky IX kultivieren.

Blumenberg und Wiening,[7] Klemt,[8] Bogen und Benett,[9] Petroff und Steenken,[10] Winterberg,[11] Jordan,[12] Mikami[13] u.a. zählten die Tuberkelbazillen in 1 mg Masse. Wir haben nun vorliegende Untersuchungen durchgeführt, um festzustellen, wieviel Tuberkelbazillen in Sputum, Magensaft, Gurgelwasser, Exsudat usw. vorhanden sein müssen, um durch Kultur nachgewiesen werden zu können.

II. Zählung- und Kulturmethode der Tuberkelbazillen.

1. *Zählung der Tuberkelbazillen.*

Zu den Versuchen dienten auf Lockemannscher Nährflüssigkeit 3 Wochen lang kultiveierte, gut gedeihende Tuberkelbazillen des Typus humanus. Nachdem man sie mit trocknem, sterilisiertem Fillterpapier hinreichend entwässert und ihr Gewicht bestimmt hatte, stellte man unter Zusatz steriler physiologischer Kochsalzlösung durch zerreiben im Achatmörser eine Emulsion von 1 mg Tuberkelbazillen pro 1 ccm her. Man verdünnte sie 10 fach und berechnete dann die enthaltenen Bakterien ebenso wie Mikami[13] mit Hilfe der Reichert schen Bazillenzählkammer.

2. *Kulturmethode der Bazillen-Kontrollemulsion.*

Zuerst wurde eine Emulsion von 1 mg Tuberkelbazillen des Typus humanus pro ccm 0,9% iger steriler physiologischer Kochsalzlösung hergestellt. Durch Verdünnung wurden aus dieser vor jedem Versuche Emulsionen von $\frac{1}{10{,}000}$ mg, $\frac{1}{100{,}000}$ mg, $\frac{1}{1{,}000{,}000}$ mg und $\frac{1}{10{,}000{,}000}$ mg Bazillen pro ccm hergestellt. Jeweils 0,05 ccm davon kamen auf 5–20 Oka-Katakurasche Phosphorsaüresalz-Azinomoto-Eier-Nährböden. Aus den herausgezüchteten Kolonien berechnete man die Kolonienzahlen auf je 1 ccm Bakterienaufschwenmmug. Aus den viermal wiederholten Versuchen ergab sich, dass 1 mg Bazillenmasse 140,000,000, 150,000,000, 127,000,000, 270,000,000, also durchschnittlich 166,000,000 Tuberkelbazillen des Typus humanus enthält. Mithin beträgt die Bazillenzahl in $\frac{1}{10{,}000}$ mg, $\frac{1}{100{,}000}$ mg, $\frac{1}{1{,}000{,}000}$ mg und $\frac{1}{10{,}000{,}000}$ mg Bakterienmasse jeweils 12,706–27,000, 1,270–2,700, 127–270 bzw. 12–27, durchschnittlich also 16,625, 1,663, 166 bzw. 21. Die Kolonienzahl beträgt 186–556, 52–96, 4–12 bzw. 0–1, durchschnittlich also 310, 80, 8 bzw. 1. Die Bazillenzahl für eine Kolonie beläuft sich bei der Konzentration von $\frac{1}{10{,}000}$ mg auf 48–68, durchschnittlich 54, bei der von $\frac{1}{100{,}000}$ mg auf

16–28, durchschnittlich also 21, bei der von $\frac{1}{1{,}000{,}000}$ mg auf 12–27, durchschnittlich 20 und bei der von $\frac{1}{10{,}000{,}000}$ mg, durchschnittlich 23. Alwo bilden bei der Emulsion von $\frac{1}{10{,}000}$ mg 50 und bei denen von $\frac{1}{100{,}000}$ — $\frac{1}{10{,}000{,}000}$ mg 24–20 Tuberkelbazillen eine Kolonie.

TABELLE 1.

Beziehungen zwischen Bazillen- und Kolonienzahl in Emulsionen von Tuberkelbazillen (Typus humanus).

Bazillenmenge (mg)	Versuch I			Versuch II			Versuch III		
	Bazillenzahl	Kolonien	Bazillenzahl pro eine Kolonie	Bazillenzahl	Kolonien	Bazillenzahl pro eine Kolonie	Bazillenzahl	Kolonien	Bazillenzahl pro eine Kolonie
1	140,000,000			150,000,000			127,000,000		
$\frac{1}{10{,}000}$	14,000	204	68	15,000	296	50	12,000	186	68
$\frac{1}{100{,}000}$	1,400	84	16	1,500	88	17	1,200	52	24
$\frac{1}{1{,}000{,}000}$	140	8	17	150	12	12	120	4	31
$\frac{1}{10{,}000{,}000}$	14	1	14	15	0		12	0	

Bazillenmenge (mg)	Versuch IV			Durchshnitt		
	Bazillenzahl	Kolonie	Bazillenzahl pro eine Kolonie	Bazillenzahl	Kolonie	Bazillenzahl pro eine Kolonie
1	270,000,000			166,250,000		
$\frac{1}{10{,}000}$	27,000	556	48	16,625	310	54
$\frac{1}{100{,}000}$	2,700	96	28	1,662	80	21
$\frac{1}{1{,}000{,}000}$	270	10	27	166	8	20
$\frac{1}{10{,}000{,}000}$	27	1	27	68/4	2/4	23

III. Genauigkeit der Kulturmethode zum Nachweis der Tuberkelbazillen aus Sputum.

Zuerst wurde keine Tuberkelbazillen enthaltendes Sputum im Mörser gut und homogen zerrieben. Dann wurden hiervon 0,5 1, 2, 5 und 10 ccm in sterilisierten Zentrifugalgläschen nach Zusatz von 14,000, 1,400, 140 und 14 Tuberkelbazillen des Typus humanus gut gemischt.

Endlich wurden die Proben mit der ungefähr 4–7 fachen Menge 4%iger Schwefelsäure versetzt und nochmals mit einem sterilisierten Stäbchen tüchtig umgerührt und zerrieben. Nach 20–30 Minuten langem Stehen bei Zimmertemperatur wurden sie 10 Minuten lang mit 3,000 Umdrehungen pro Minute zentrifugiert und die überstehende Flüssigkeit abgegossen. Je 3 Ösen der Niederschläge wurden auf 5 Oka-Katakurasche Phosphorsäuresalz-Azinomoto-Eier-Nährböden gebracht, 2 Monate lang im Thermostaten bei 37°C aufbewahrt. Dabei wurden am 15., 30., 40. und 60. Tage die entstandenen Kolonien gezählt. Um die Versuchsfehler möglichst zu verringen, wurden dieselben Versuche viermal durchgeführt und aus ihnen die Mittelwerte gezogen.

Die durchschnittliche Bazillenzahl in 1 ccm Stammemulsion von 1 mg Bakterienmasse pro 1 ccm ist 140,000,000. Mithin beträgt sie bei den verdünnten Emulsionen von $\frac{1}{10,000}$ mg, $\frac{1}{100,000}$ mg, $\frac{1}{1,000,000}$ mg und $\frac{1}{10,000,000}$ mg Bazillenmasse pro 1 ccm 14,000, 1,400, 140 bzw. 14. Aus 1 ccm dieser verdünnten Aufschwemmungen wurden 204, 84, 8 bzw. 1 Kolonien herausgezüchtet. Eine Kolonie entspricht 68, 16, 17 bzw. 14 Tuberkelbazillen. Wenn man in 0,5 ccm, 1ccm, 2 ccm, 5 ccm und 10 ccm Sputum 14,000, 1,400, 140 und 14 Tuberkelbazillen einführte und

TABELLE 2.

Resultate und Empfindlichkeit der Kultur von Tuberkelbazillen aus Sputum.

Stammemulsion in 1 ccm					Menge des Sputum					
					0,5 cc			1 cc		
Bazillenmenge (mg)	Bazillenzahl	Kolonie	Bazillenzahl pro eine Kolonie	Empfindlichkeit	Kolonie	Bazillenzahl pro eine Kolonie	Empfindlichkeit	Kolonie	Bazillenzahl pro eine Kolonie	Empfindlichkeit
1	140,000,000									
$\frac{1}{10,000}$	14,000	204	68	0,014	40	350	0,003	58	241	0,004
$\frac{1}{100,000}$	1,400	84	16	0,060	18	77	0,013	37	38	0,026
$\frac{1}{1,000,000}$	140	8	17	0,057	1	140	0,007	1	140	0,016
$\frac{1}{10,000,000}$	14	1	14	0,071	0			0		
Kontroll Sputum										

Je 3 Ösen des durch Zentrifugieren aus Sputum gewonnenn Bodensatzes werden auf einem Nährboden gezüchtet

TABELLE 2.

(*Fortgesetzt*)

Stammemulsion in 1 cc	Menge des Sputum								
	2 cc			5 cc			10 cc		
Bazillenmenge (mg)	Kolonie	Bazillenzahl pro eine Kolonie	Empfindlichkeit	Kolonie	Bazillenzahl pro eine Kolonie	Empfindlichkeit	Kolonie	Bazillenzahl pro eine Kolonie	Empfindlichkeit
1									
$\frac{1}{10,000}$	100	140	0,007	93	150	0,006	35	400	0.002
$\frac{1}{100,000}$	38	36	0,027	11	127	0,008	4	350	0,003
$\frac{1}{1,000,000}$	2	70	0,016	1	140	0,007	0		
$\frac{1}{10,000,000}$	0			0			0		
Kontroll Sputum									

Je 3 Ösen des durch Zentrifugieren aus Sputum gewonnenen Bodensatzes werden auf einem Nährboden gezüchtet

kultivierte, so bildeten sich jeweils 40, 58, 100, 93, 35; 18, 37, 11, 4: 1, 1, 2, 1, 0; sowie 0,0,0, 0,0, Kolinien. Eine Kolonie entspricht bei 0,5 ccm Sputum 350, 77, 140, bei 1 ccm Sputum 241, 38, 140, bei 2 ccm Sputum 140, 36, 70, bei 5 ccm Sputum 150, 127, 140 und bei 10 ccm 400, 350 Bakterien. Das Minimum der Bazillenzahl, welche man als Kontrolle für 1 ccm Stammemulsion kultivieren kann, beträgt 14 und die Empfindlichkeit der Kultur 0,071. Die kleinste Zahl der Krankheitserreger, die man in 2 ccm Sputum kulturell nachweisen kann, beträgt aber 38 und die Empfindlichkeit der Kultur 0,017. Die Empfindlichkeit der Kultur nennen wir vorläufig, Kolonienzahl dividiert durch geimpfte Bazillenzahl, also die aus einem Bakterium zu bildende Kolonienzahl.

IV. Genauigkeit der Kulturmethode der Tuberkelbazillen aus Magensaft

Die Versuche wurden an tuberkulinnegativen, nicht-tuberkulösen Patienten durchgeführt. Am frühen Morgen wurde vor der Nahrungsaufnahme eine sterilisierte Magensonde eingeführt und Magensaft (ungefähr 40 ccm) mittelst einer Spritze entnommen. Konnte man keinen Magensaft erhalten, wurde der Magen mit sterilem, destilliertem Wasser gespült. 20 ccm des so gewonnenen Magensaftes oder Spülwassers wurden nach Zusatz einer bestimmten Anzahl humaner Tuberkelbazillen gut gemischt; die verbleibenden Reste des Magensaftes oder Spülwassers

dienten Kontroll-kulturen. Den Versuchsmaterialien setzte man zunächst zur Auflösung des Schleimes 10 ccm 2% iger NaOH-Lösung danach bis zum leichten Opaleszierien 2,5% ige $ZnSO_4+7H_2O$-Lösung zu. Der so erhaltene Niederschlag wurde 10 Minuten lang mit 3,000 Umdrehungen pro Minute zentrifugiert, die überstehende Flüssigkeit abgegossen. Dann wurde nach Zugabe von 10 ccm 4%iger H_2SO_4 5 Minuten lang mit 3,000 Umdrehungen pro Minute zentrifugiert. Je 3 Ösen dieser Neiderschläge wurden auf 5 Oka-Katakura sche Nährboden gestrichen. Die Kulturen wurden ebenso wie in den Sputum-Versuchen geprüft.

Um die Versuchsfehler möglichst zu verringern, wurden hier ebenfalls dieselben Versuche viermal wiederholt und aus ihnen die Mittelwerte gezogen.

1 mg Bazillenmasse in 1 ccm Stammemulsion enthält 150,000,000 Bakterien. Folglich beträgt die Bazillenzahl in $\frac{1}{10,000}$ mg, $\frac{1}{100,000}$ mg, $\frac{1}{1,000,000}$ mg und $\frac{1}{10,000,000}$ mg Bakterienmasse 15,000, 1,500, 150 bzw. 15, und die Kolonienzahl beträgt 296, 88, 12 bzw. O. Eine Kolonie entspricht 56, 17 zbw. 12 Tuberkelbazillen. Wenn man diesen Bakterienaufschwemmungen 20 ccm Magensaft zufügte und dann kultiveierte, so entstanden 80, 24, 2 bzw. O Kolonien. Eine Kolonie entspricht dabei 187, 62 bzw. 70 Tuberkelbazillen.

TABELLE 3.

Resultate und Empfindlichkeit der Kultur von Tuberklbazillen aus Magensaft.

	Stammemulsion in 1 cc				Menge des Magensaft 20 cc		
Bazillenmenge	Bazillenzahl	Kolonie	Bazillenzahl pro eine Kolonie	Empfindlichkeit	Kolonie	Bazillenzahl pro eine Kolonie	Empfindlichkeit
1	150,000,000						
$\frac{1}{10,000}$	15,000	296	50	0,019	80	187	0,005
$\frac{1}{100,000}$	1,500	88	17	0,058	24	62	0,016
$\frac{1}{1,000.000}$	150	12	12	0,080	2	70	0,013
$\frac{1}{10,000,000}$	15	0	0		0	0	
	Kontroll-Magensaft				0		

Je 3 Ösen des durch Zentrifugieren aus Magensaft gewonnenen Bodensatzes werden aus einem Nährboden gezüchtet.

Bei 20 ccm Kontroll-Magensaft fiel die Tuberkelbazillen-Kultur negativ aus. Das Minimum der Bakterienzahl, welche man aus 1 ccm Stammemulsion herauszüchten kann, beträgt also 12, und die Empfindlichkeit der Kultur 0,08. Die wenigsten Bazillen, die man aus 20 ccm Magensaft herauszüchten kann, sind dagegen 62 und die Empfindlichkeit der Kultur ist 0,016.

V. Genauigkeit der Kulturmethode zum Nachweis der Tuberkelbazillen aus Gurgelwasser

Die Versuche wurden ebenfalls an tuberkulinnegativen, nicht-tuberkulösen Kranken angestellt. Am frühen Morgen liess man die Versuhspersonen vor Reinigung der Mundhöhle mit 40 ccm aseptischem Wasser gut gurgeln, um den Schleim von der hinteren Rachenwand abzuspülen, und das Gurgelwasser in ein grosses Reagenzglas von 100 ccm Inhalt ausgiessen. 20 ccm davon wurden nach Zugabe einer bestimmten Menge Tuberkelbazillen tüchtig umgerührt und gemischt. Die vorbleibenden Reste des Spülwassers wurden zu den Kontrollversuchen benutzt. Nach spontaner Niederschlagung nahm man ungefähr 10 ccm Bodensatz und gab dann die gleiche Menge 4%ige H_2SO_4 hinzu. Nach 20 Minuten langem Stehenlassen wurde das Versuchsmaterial 10 Minuten lang mit 3,000 Umdrehungen pro Minute zentrifugiert und mit demselben Verfahren wie bei den Sputum-Versuchen kultiviert. Die Kulturen wurden ebenfalls nach der gleichen Methode geprüft. Aus den Versuchen ergab

TABELLE 4.

Resultate und Empfindlichkeit der Kultur von Tuberkelbazillen aus Spülwasser.

	Stammemulsion in 1 cc				Menge des Spülwasser 20 cc		
Bazillenmenge	Bazillenzahl	Kolonie	Bazillenzahl pro eine Kolonie	Empfindlichkeit	Kolonie	Bazillenzahl pro eine Kolonie	Empfindlichkeit
1	127,000,000						
$\frac{1}{10,000}$	12,700	186	68	0,014	72	176	0,005
$\frac{1}{100,000}$	1,270	52	24	0,040	14	90	0,011
$\frac{1}{1,000,000}$	127	4	31	0,031	0		
$\frac{1}{10,000,000}$	13	0			0		
Kontrol-Spülwasser					0		

Je 3 Ösen des durch Zentrifugieren aus spülwasser gewonnenen Bodensalzes werden auf einem Nährboden gezüchtet.

sich, dass die geringste Anzahl Bazillen, die sich aus 20 ccm Spülwasser herauszüchten lässt, 90 und die Empfindlichkeit der Kutlur 0,01 beträgt während in den Kontrollversuchen mit verdünnten Bakterienaufschwemmungen eine Kolonie 68, 24 bzw. 31 Tuberkelbazillen entspricht.

VI. Genauigkeit der Kulturmethode zum Nachweis der Tuberkelbazillen aus Exsudat.

Diese Versuchsreihe wurde an Pleuritikern durchgeführt, und klares Exsudat, in welchem mikroskopisch keine Tuberkelbazillen nachweisbar waren, zu den Versuchen verwendet. Je ungefähr 20 ccm Exsudat wurden in sterilisierten Zentrifugalgläschen mit einer bestimmten Menge Tuberkelbazillen versetzt. Nach Zugabe von 20 ccm sterilem, destilliertem Wasser wurden sie tüchtig umgerührt und gemischt und dann 24 Stunden lang im Thermostaten aufbewahrt. Nach Durchführung der Tomita[14] schen Fibrinmethode wurden sie 10 Minuten lang mit 3,000 Umbdrehungen pro Minute zentrifugiert und dann auf je 5 Oka-Katakura schen Nährböden kultiviert. Die Kulturen wurden ebenso wie bei den Sputum-Versuchen geprüft. 1 mg Bazillenmasse humaner Tuberkelbazillen enthält 270,000,000,000 Bazillen: mithin beträgt die Bazillenzahl in $\frac{1}{10{,}000}$ mg, $\frac{1}{100{,}000}$ mg, $\frac{1}{1{,}000{,}000}$ mg und $\frac{1}{10{,}000{,}000}$ mg Bazillenmasse 27,000, 2,700, 270 bzw. 27. Die Kolonienzahl beträgt 556, 96, 10 bzw. 1. Aus 20 ccm Kontroll-Exsudat wurden 7 Kolonien heraus gezüchtet.

TABELLE 5.

Resultate und Empfindlichkeit der Kultur von Tuberkelbazillen aus Exsudat.

	Stammemulsion in 1 ccm				Menge des Exsudates 20 cc		
Bazillenmenge (mg)	Bazillenzahl	Kolonie	Bazillenzahl pro eine Kolonie	Empfindlichkeit	Kolonie	Bazillenzahl pro eine Kolonie	Empfindlinchkeit
1	270,000,000						
$\frac{1}{10{,}000}$	27,000	556	48	0,026	124—7=117	230	0,004
$\frac{1}{100{,}000}$	2,700	96	28	0,035	60—7=53	50	0,019
$\frac{1}{1{,}000{,}000}$	270	10	27	0,037	14—7=7	38	0,026
$\frac{1}{10{,}000{,}000}$	27	1	27	0,028	8—7=1	27	0,037
Kontroll-Exsudat					7		

Je 3 Ösen des durch Zentrifugieren aus Exsudat gewonnenen Bodensatzes werden auf einem Nährboden gezüchtet.

Die kleinste Bazillenmenge, welche man zur Kontrolle für 1 ccm Stammemulsion herauszüchten kann, beträgt 27, und die aus Exsudat herauszüchtbare ebenfalls 27.

VII. Zusammenfassung.

Bei der Kultur von Tuberkelbazillen denkt man leicht, alle Bazillen der zu untersuchenden Materials bildeten Kolonien. Wenn also die Kultur aus den Sekreten des Patienten negativ ausfällt, so glaubt man leicht, dass er nicht an Tuberkulose leide oder schon von ihr genesen sei. Bei der Kultur so schwer zu züchtender Bazillen wie der Tuberkelbazillen bilden aber möglicherweise nicht alle lebenden Bazillen Kolonien.

Dazu kommt noch, dass die im zu untersuchenden Material enthaltenen Bazillen nicht alle leben. Darum bildet bei der Kultur nur ein Teil von ihnen Kolonien. Bei der Kultur von Bakterienaufschwemmungen kann man den grössten Teil der Bazillen auf den Nährboden bringen. Bei der anderer Untersuchungsmaterialien aber, die mit Säure oder Alkali vorbehandelt werden müssen, wird ein Teil der in ihnen enthaltenen Bakterien abgetötet. Wenn man also daran denkt, dass nur ein Teil der enthaltenen Bazillen durch das Zentrifugieren zur Fällung kommt und dass wiederum nur ein Teil der in diesem Niederschlag enthaltene Bakterien kultiviert wird, so erkennt man, dass nur ein sehr geringer Teil der im zu untersuchenden Material enthaltenen Bazillen durch Kultur nachweisbar ist. Diese Tatsache ist in unseren Versuchen bestätigt worden, aus denen sich ergab, dass bei der Kultur von Aufschwemmungen humaner Tuberkelbazillen im grossen ganzen von 20–50, bei der aus Sputum dagegen von 40–400 und bei der aus Magensaft oder Magenspülwasser 62–187 bzw. 90–176 Bakterien eine Kolonie gebildet wird. Dass bei der Kultur aus Exsudat verhältnismässig wenige Bazillen schon eine Kolonie bildeten, beruhte wahrscheinlich darauf, dass infolge der Wirkung des Fibrins die Bakterien verhältnismässig leicht gesammelt werden konnten.

Aus den Resultaten unserer Versuche ersieht man, dass die Kultur als Nachweismethode der Tuberkelbazillen, wenn auch schon ziemlich fortgeschritten doch noch verbesserungsbedürftig ist.

VIII. Schluss.

Wir haben Emulsionen von Tuberkelbazillen des Typus humanus hergestellt und mittelst der Reichertschen Rechentafel die in ihnen enthaltenen Krankheitserreger gezählt. Gleichzeitig haben wir sie auf den Oka-Katakura schen Nährböden kultiveirt und die auf diesen herausgezüchteten Kolonien gezählt, um das Verhältnis zwischen Bazillenzahl und Kolonienbildung zu untersuchen. Andererseits haben wir Tuberkelbazillen in bestimmten Mengen zu Sputum, Magensaft, Gurgel-

wasser, Exsudat usw. hinzugefügt und in der klinisch üblichen Weise auf Oka-Katakura schen Nährböden kultiviert, um ebenfalls das Verhältnis zwischen Bazillenzahl und Kolonienbildung zu untersuchen.Aus den Versuchen ergaben sich folgende Resultate:

1) Bei Kultur einer Aufschwemmung von $\frac{1}{10{,}000}$ mg Bazillenmasse pro 1 ccm bilden 48–68, durchschnittlich also 54 Bazillen eine Kolonie. Bei der Konzentration von $\frac{1}{100{,}000}$ mg und $\frac{1}{1{,}000{,}000}$ mg bilden 12–31 durchschnittlich 20 Bazillen eine Kolonie.

2) Wenn man zu Sputum Tuberkelbazillen hinzufügt, mit Schwefelsäure behandelt und 3 Ösen des durch Zentrifugieren entstandenen Bodensatzes kultiviert, so bilden bei Zusatz von $\frac{1}{10{,}000}$ mg Bazillenmasse 140-400, bei dem mit $\frac{1}{100{,}000}$—$\frac{1}{1{,}000{,}000}$ mg 35–350 Bazillen eine Kolonie. Bei der Zugabe von $\frac{1}{10{,}000{,}000}$ mg ist der Nachweis von Tuberkelbazillen unmöglich.

3) Wenn man Tuberkelbazillen in 20 ccm Magensaft oder Gurgelwasser einführt, so bilden bei Hinzufügung von $\frac{1}{10{,}000}$ mg Bazillenmasse 170–190, bei der von $\frac{1}{100{,}000}$—$\frac{1}{1{,}000{,}000}$ mg 60–90 Bazillen eine Kolonie.

4) Wenn man Tuberkelbazillen zu 20 ccm Exsudat hinzufügt, so bilden bei Zugabe von $\frac{1}{10{,}000}$ mg Bazillenmasse 230, bei der von $\frac{1}{100{,}000}$ —$\frac{1}{1{,}000{,}000}$ mg 38–50, bei der von $\frac{1}{10{,}000{,}000}$ mg 27 Bazillen eine Kolonie.

Diese Untersuchungen konnten durch Überlassung von Geldmittln des Unterrichtsministeriums für wissenschaftliche Forschung ausgeführt werden, wofür herzlich gekankt sei. Prof. Dr. T. Kumagai und
Prof. Dr. T. Ebina.

Literatur.

(1) Kumagai, Nihon Rinsyo Kekkaku, 1940, **1**, 1.
(2) Kumagai, Haikekkaku no Sokisindan to Tiryosisin, 1940, Kanahara Tokio.
(3) Butschowitz, Z. Tbk., 1931, **60**, 149.
(4) Iibuti, TokyoIzisinsi, 1933, **2837**, 1627.
(5) Katakura u. Oka, Tohoku Igaku Zassi, 1939, **24**, 71.
(6) Oakda, Nihon Rinsyo Kekkaku, 1942, **3**, 849.
(7) Blumenberg u. Wiening, Z. Tbk., 1928, **51**, 417.
(8) Klemt, Kekkaku, 1940, **18**, 997.

(9) Bogen a. Benett, Amer. Rev. Tbc., 1939, **39**, 89.
(10) Petroff u. Steenken, Proc. Soc. exp. Biol. Med., 1927, **24**, 958.
(11) Winterberg, Z. Hyg., 1897, **29**, 75.
(12) Jordan, Amer. Rev. Tbc., 1938, **38**, 241.
(13) Mikami, Nihon Rinsyo Kekkaku, 1944, **5**, 290.
(14) Tomita, Beitr. kl. Tbk., 1938, **92**, 422.

The Three Color Processes in the Retina of Frog.

By

Koiti Motokawa and **Kituya Iwama.**

(本川 弘一)　　(岩間 吉也)

(From the Physiological Laboratory of Prof. K. Motokawa, Tohoku University, Sendai.)

(Received for publication, April 20, 1949)

The electrical excitability of excised frog eyes following two seconds' illumination by white or colored light was studied, taking the least perceptible impulses of the optic nerve set up by electrical stimulation of the eye as the index of retinal excitation. The stimulating current was a constant current pulse of one second in duration. Examples of the excitability curves which represent the time course of excitability changes following illumination are illustrated in Fig. 1. The curve for white light shows three distinct humps which may be denoted by R, G and B (see (a) in Fig. 1). The time to the maximum or the crest time of each hump remains unaltered as the intensity of light is varied over a wide range. This finding suggests that in the retina of frog, there are three kinds of processes with different time constants. The three processes are elicited in different proportions according to the wave-length of light just as the trichromatic theory advocates. Fig. 1 (b) represents the excitability curves for red light, which provokes the R process greatly, and the G process only slightly, but cannot elicit the B process.

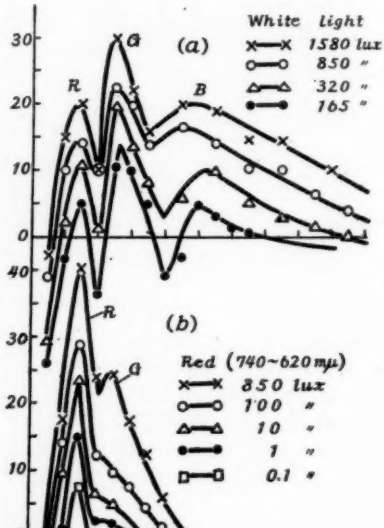


Fig. 1. Ordinate: percentage increase of electrical excitability.

Abscissa: time from the end of illumination to electrical stimulation.

The Tohoku Journal of Experimental Medicine, Vol. 50, Nos. 3 & 4, 1949.

Ueber die entwicklungshemmende Wirkung des Blutes von verschiedenen Tieren auf Tuberkelbazillen.

von

Kozo Otomo.

(大友 孝藏)

(Aus dem Forschungsinstitut für Krankheiten der Säurefestbazillen an der kaiserlichen Tohoku-Universität zu Sendai. Vorstand: Prof. Dr. T. Kumagai.)

(Received for publication, May 7, 1946)

I. Einlentung.

Es ist nicht nur den Veterinären bekannt, sondern auch experimentell schon nachgewiesen, dass Tiere wie Pferd, Hund, Katze, Vogel, Ratte, Maus usw. für Tuberkelbazillen des humanen Typus schwer empfänglich sind. Seitdem die neue Methode der Slide Cell Culture im Jahre 1924 von A.E. Wright[1] als eine der Beobachtungsmethoden des Wachstums der Tuberkelbazillen im Blut veröffentlicht wurde, sind vergleichende Studien über die entwicklungshemmende Wirkiung auf Tuberkelbazillen im Blut verschiedener schwer infektiöser Tiere von mehreren Forschern nach dieser Methode lebhaft aufgenommen worden. Sie haben sich dabei bemüht, die wahre Ursache der Unempfänlgichkeit in ihrem Blut zu erfassen. Bei uns in Japan untersuchten Yabusita,[2] Miwa[3] u.a. im Vollblut von Meerschweinchen, Kaninchen, Ratte usw. die entwicklungshemmende Wirkung auf humane Tuberkelbazillen durch die Slide Cell Culture. Sie stellten fest, dass die entwicklungshemmende Wirkung des Vollblutes auf Bazillen, abgesehen von mehr oder weniger oft vorkom mender experimenteller Schwankung, sich in der Reihenfolge von Meerschweinchen, Kaninchen und Ratte erhöht. Sato[4] teilte mit, dass das Wachstum humaner Tuberkelbazillen im Vollblut der Ratte und des Huhnes nicht nachweisbar ist und dass es auch im Falle des Kaninchens bei 5 von 8 Versuchstieren negativ ausfiel. Er führte diese Tatsache auch nicht auf die im Vollblut vorhandene bakterizide, sondern auf seine bakteriostatische Wirkung zurück, weil er die für die Slide Cell Culture verwandten Tuberkelbazillen, die er weiter in anderen Nährboden versetzte oder anderen Tieren einimpfte, lebend vorfand.

Auch Ito[5] stellte fest, dass das Wachstum humaner Tuberkelbazillen

im Vollblut der Ratte und der Ziege nicht nachweisbar ist und auch beim Hund die hemmende Wirkung äusserst auffallend ist. Er stellte auch fest, dass beim Meerschweinchen zwischen der entwicklungshemmenden Wirkung auf Tuberkelbazillen im Plasma und der im Vollblut kein Unterschied vorliegt. Dadurch kam er auf den Schluss, dass diese entwicklungshemmende Wirkung auf Bazillen nicht durch die Phagozytose der Leukozyten verursacht wird, wie es Wright behauptete, sondern vielmehr im Blutplasma zu suchen ist. Horai[6] unternahm die-Kultur humaner Tuberkelbazillen durch Slide Cell Culture in bezug auf das Vollblut und Plasma des gesunden Meerschweinchens, Kaninchens und Menschen und teilte mit, dass die entwicklungshemmende Wirkung in den ersten zwar nachwiesbar, doch im letzteren merklicher ist. Uesaka[7] stellte Kirchner schen Nährboden her, dem er Blutserum von Meerschweinchen, Kaninchen, Huhn und Tuberkulösen im Verhältnis von je 10% zusetzte. Er nahm damit die Tiefenkultur humaner Tuberkelbazillen vor, um den Wachstumszustand der Bazillen zu beobachten. Vom Standpunkt aus, dass jedes Serum eine günstige Wirkung auf die Entwicklung der Tuberkelbazillen ausübt, kam er auf den Schluss, dass der Unterschied der Empfänglichkeit jedes Versuchstiers für Tuberkelbazillen ausserhalb des Serums zu suchen ist. So sehen wir, dass die entwicklungshemmende Wirkung auf Tuberkelbazillen zwar bei verschiedenen schwer emfpänglichen Tieren nachweisbar, doch ihre Stärke nicht nur unbestimmt ist, sondern auch die Meinungen über das Wo des wahren hemmenden Stoffs geteilt sind: ob er im Vollblut, im Serum oder im Plasma zu suchen ist.

Kürzlich habe ich nun nach der Methode der Slide Cell Culture die entwicklungshemmende Erscheinung gegen Tuberkelbazillen einerseits bezüglich des Vollblutes von Ratte, Hund und Pferd, die als schwer emfänglich für humane Tuberkelbazillen gelten, anderseits bezüglich des vom relativ empfänglichen Meerschweinchen verglichen. Nebenbei habe ich nach der Kirchner schen Tiefenkultur die Beobachtung der entwicklungshemmenden Wirkung auf Bazillen des humanen Typus angestellt. Die gewonnennne Ergebnisse werden im folgenden mitgeteilt.

II. Vergleich der entwicklungshemmenden Wirkung des Vollblutes von verschiedenen Tieren auf Tuberkelbazillen.

1) *Methode*

Was die Versuchsmethode der Slide Cell Culture betrifft, so richtete sie sich nach der Miyasaka[9]schen Methode, einer modifizierten Wrightschen Methode. Das geänderte Verfahren war folgendes: die gewählten humanen Tuberkelbazillen waren solche, die 3 Wochen lang in der Phosphat- Natr. glutaminicum-Glycerin-Lösung gezüchtet worden waren.

Ihre Bestandteile:

KH_2PO_4	4,0 g
Na_2HPO_4	3,0 g
Natr. glutaminicum	5,0 g
Natr. citricum neutral	2,5 g
Magn. sulfuricum	0,6 g
Glycerin	20,0 ccm
Aq. destillata	1000,0 ccm

Was die homogene Bazillenaufschwemmung anbetrifft, so wurde sie nach genauer Wägung so hergestellt, dass 1 mg der durch sterilisiertes Filterpapier wasserfrei gewordenen Bazillen in 1,0 ccm sterilisierter physiologischer Kochsalzlösung enthalten sind. Sie wurden 5 Minuten lang mit der Zentrifuge bei einer Umdrehungszahl von etwa 3000 pro Minute zentrifugiert. Der obere klare Teil wurde je 0,1 ccm in sterile Reagenzgläser gegossen, dem dann je 1,0 ccm Tierblut beigefügt wurde. Beide wurden nun, nachdem sie schnell und vollständig gemischt worden waren, in Slide Cell hineingegossen und gezüchtet. Was das Entnehmen des Blutes anbetrifft, so wurde es bei der Ratte und dem Meerschweinchen durch Herzpunktion, beim Kaninchen von den hinteren Auricularvenen, beim Pferde von den Halsvenen und zwar in allen Fällen mit der Injektionsspritze entnommen. Als Kontrolle standen dieselben gesunden Kaninchen, deren Tuberkulinreaktion negativ war, wiederholt zur Verfügung.

2) *Versuchsergebnisse.*

Wie in der Tabelle I ersichtlich ist, ist die entwicklungshemmende Wirkung auf Tuberkelbazillen im Rattenblut am stärksten; denn selbst am 9. Tage der Kultur lässt sich noch kein Wachstum der Bazillen erkennen, und erst am 11. Tage konnte man nur mit Mühe eine geringfügige Entwicklung derselben wahrnehmen.

Obwohl man auch bei Hund und Pferd, individuellen Unterschied ausser acht gelassen, im allgemeinen eine merkliche hemmende Wirkung auf Tuberkelbazillen erkennt, so ist doch der Grad der betreffenden Wirkung unvergleichlich schwächer als bei der Ratte. Beim Meerschweinchen zeigte sich ein besserer Entwicklungszustand als beim Kaninchen-blut.

Wenn man nun den Wachstumsgrad der Bazillen im Vollblut des Kaninchens mit 10 bezeichent, so ist er bei der Ratte 0,8, beim Hunde 6,8, beim Pferde 6,9und beim Meerschweinchen 10,4.

Obwohl, wie ersichtlich, die entwicklungshemmende Wirkung auf Bazillen in der Reihenfolge von Ratte, Hund, Pferd, Kaninchen und Meerschweinchen allmählich abnimmt, so kann man einerseits zwischen Hund und Pferd, anderseits zwischen Kaninchen und Meerschweinchen

keinen merklichen Unterschied erkennen.

Tabelle I.

Vergleich der entwicklungshemmenden Wirkung des Vollbluts von verschiedenen Tieren auf Tuberkelbazillen.

Tierenname und Nummer		Entwicklungszustand				Wachstumsgrad
		5 Tage	7 Tage	9 Tage	11 Tage	
Hund	1	−	+	++	+++	6,8
	3	−	++	++	+++	
	4	−	+	++	++	
	7	−	+	++	++	
	10	−	+	++	+++	
Kontrolle		−	++	+++	+++	10,0
Ratte	31	−	−	−	±	0,8
	34	−	−	−	−	
	35	−	−	−	±	
Kontrolle		±	±	+	+++	10,0
Pfred	1	±	±	+	++	6,9
	2	±	+	++	++	
	3	±	±	+++	+++	
	4	−	+	++	++	
	5	−	±	+	+	
	6	±	+	+++	+++	
	7	−	±	+	++	
	8	−	−	+	++	
	9	±	+	++	+++	
	10	−	+	+	+	
Kontrolle		±	−	++	+++	10,0
Meerschweinchen	222	±	+	+++	+++	10,4
	228	±	++	+++	+++	
	230	±	+	+++	+++	
	231	±	+	+++	+++	
	239	±	+	++	+++	
	240	±	++	++	+++	
	244	±	+	++	+++	
	245	±	++	+++	+++	
	259	±	+	++	+++	
	260	±	±	+++	+++	
Kontrolle		±	+	+++	+++	10,0

Bemerkung:

−: Bazillenentwicklung negativ.

±: Die meisten der Kolonien bestehen aus 2–3 Bazillen.

+: Die meisten der Kolonien bestehen aus 6–10 Bazillen.

++: Die meisten der Kolonien bestehen aus 11–30 Bazillen.

+++: Die meisten der Kolonien bestehen aus 31–50 Bazillen

++++: Die meisten der Kolonien bestehen aus mehr als 50 Bazillen.

III. Vergleich der entwicklungshemmenden Wirkung auf Tuberkelbazillen im Serum und Plasma.

1) *Methode.*

Von der oben erwähnten Phosphat-Natr. glutaminicum-Glycerin-Lösung wurden je 2,0 ccm in mit Watte zugepfropfte Reagenzgläser von 25 ccm Kapazität gegossen. Um synthetischen Nährboden zu bereiten, fügte man für die Serumreihe Serum im Verhältnis von 20%, für die Plasmareihe Plasma im Verhältnis von 50% hinzu. Beiden gab man je 0,1 ccm homogene Bazillenaufschwemmung bei, die so hergestellt war, dass in 1,0 ccm sterilisierter physiologischer Kochsalzlösung 0,5 mg Bazillen enthalten waren. Damit nahm man die Tiefenkultur vor, um den Entwicklungszustand der Tuberkelbazillen vergleichend zu beobachten.

TABELLE II.

Die entwicklungshemmende Wirkung des Serums auf Tuberkelbazillen.

Tierenname und Nummer		Slide Cell Culture		Tiefenkultur			
		7 Tage	11 Tage	10 Tage	20 Tage	30 Tage	40 Tage
Hund	1	+	###	−	±	+	++
	3	+	###	+	+	++	++
Kontrolle		++	###	+	++	###	###
Hund	4	+	###	±	+	###	###
	7	+	++	±	+	++	###
Kontrolle		++	###	++	###	###	###
Pfred	1	±	++	++	###	###	###
	2	+	++	++	###	###	###
	3	±	###	++	###	###	###
	5	±	+	±	++	###	###
	6	+	###	+	++	###	###
	7	±	++	+	++	###	###
	8	−	++	+	###	###	###
	10	+	+	+	###	###	###
Kontrolle		+	+	++	###	###	###

Bemerkung:

−: Kolonie makroskopisch nicht nachzuweisen.

±: Kolonie makroskopisch kümmerlich nachweisbar.

+: Kolonien deutlich, aber deren Anzahl klein u. Bazillenwachstum geringfügig.

++: Die Anzahl der Kolonien zwar gross doch das Bazillenwachstum geringfügig oder Bazillenentwicklung zwar deutlich, doch die Anzahl der Kolonien wenig.

###: Das Wachstum so gross, daso die Kolonien bis an die Oberfläche des Nährbodens reichten.

####: Das Wachstum sehr üppig so dass die Bazillen die Oberfläche des Nährbodens überstiegen und an die Wand der Reagenz-gläser reichten.

TABELLE III.

Die entwicklungshemmende Wirkung des Plasmas auf Tuberkelbazillen.

Tierenname und Nummer		Slide Cell Culture		Tiefenkultur			
		7 Tage	11 Tage	10 Tage	20 Tage	30 Tage	40 Tage
Hund	1	+	##	−	±	+	++
	3	+	##	+	+	++	##
Kontrolle		++	###	+	++	##	###
Hund	4	+	##	+	+	##	###
	7	+	++	±	±	##	##
Kontaolle		++	##	++	++	##	###
Pfred	1	±	++	+	+	##	###
	2	+	++	±	+	##	##
	3	±	##	++	++	##	###
	5	±	+	+	++	##	##
	6	+	##	+	+	##	###
	7	±	++	+	++	###	###
	8	−	++	+	##	##	###
	10	+	+	+	++	##	###
Kontrolle		+	###	++	##	###	###

2) *Versuchsergebnisse*

Wie in den Tabellen II und III dargestellt, gehen die Ergebnisse zwar nicht immer parallel mit dem entwicklungshemmenden Zustand, der mittels des Vollblutes durch gleichzeitig ausgeführte Slide Cell culture festgestellt wurde. Doch ist die Bazillenentwicklung sowohl beim Hunde als auch beim Pferde, wenn es sich um den mit Serum oder Plasma versehenen Nährboden handelt, merklich schlechter gegenüber derjenigen der Kontrolle. Dies ist besonders bei der Plasmareihe der Fall, bei der die hinzugefügte Menge grösser war. Wenn man ferner den Grad der entwicklungshemmenden Wirkung auf Bazillen beim Hunde mit dem beim Pferde vergleicht, so erscheint er beim ersteren etwas stärker. Und wenn man die Zeitdauer der Kultur 30 Tage überschreiten lässt und sie sich 40 Tagen nähert, so wird das Bazillenwachstum in beiden Fällen so lebhaft, dass es schwierig wird, einen Unterschied zwischen diesem Wachstum und dem der Kontrolle zu erkennen. Man konnte den Unterschied daher bedeutend besser bis zum 20 Tage, vom Beginn der Kultur angerechnet, nachweisen.

IV. Besprechung der Ergebnisse.

1) Im Vollblut von Ratte, Hund, Pferd, Kaninchen usw. wurde durch die Slide Cell culture eine deutliche entwicklungshemmende

Wirkung wahrgenommen; aber es muss zugegeben werden, dass ihr Grad im allgemeinen geringfügiger ist im Vergleich mit den in der Literatur mitgeteilten Ergebnissen von Vorgängern; nur bei der Ratte trat eine twas stärkere hemmende Wirkung hervor.

Und wie in der Mitteilung von Meissner[10], so liess sich auch hier unter Versuchstieren derselben Art ein ziemlich starker individueller Unterschied beobachten.

2) Die Tiefenkultur der Tuberkelbazillen wurde in einer Nährflüssigkeit von Phosphat-Natr. glutaminicum-Glycerin vorgenommen, der Serum oder Plasma von Hund und Pferd beigemischt war, um die entwicklungshemmende Kraft der beiden gegen humane Bazillen zu beobachten. Dabei wurde ein deutliches Vorhandensein ihrer hemmenden Wirkung erkannt. Dieses Ergebnis stimmt nicht überein mit der Ausicht von Uesaka,[6] der die entwicklungshemmende Wirkung auf Tuberkelbazillen des humanen Typus im Serum von Kaninchen, Huhn, Meerschweinchen und Tuberkulösen ablehnt. Auch mit der von Kuwahara[11] stimmt sie nicht überein, der behauptete, dass im Katzenserum keine entwicklungshemmende Wirkung nachzuweisen ist, nachdem er humane Tuberkelbazillen 24 Stunden lang im Katzenserum behandelt und diese dann Versuchstieren eingeimpft hatte. Im ersten Falle war der Kirchnersche synthetische Nährboden zum Grundnährboden gewählt worden und das Serum nur im Verhältnis von 10% hinzugemischt. Im letzteren Falle wirkten die Bazillen im Serum nur 24 Stunden lang, wenn es sich auch um reines Serum handelte. Das Ergenbis dieser beiden Experimente ist also von dem meinigen verschieden. Bei meinen Experimenten wurde die Beobachtung so angestellt wurde, dass der Prozentsatz des zum Grundnährboden zugesetzten Serums 20%, der des Plasmas 50% betrug, und die Bazillen in diesem Nährboden von relativ hoher Dichtigkeit auch längere Zeit nähmlich über 10 Tage wirken konnten. Daher kann man folgern, dass es übereilt ist, nur auf Grund der Ergebnisse der beiden Autoren ohne weiteres zu urteilen, dass die entwicklungshemmende Wirkung weder im Serum noch im Plasma vorhanden ist, weil ihnen die stark und schnell wirkende bakterizide Eigenschaft fehlt.

Ich bin der Ueberzeugung, dass es die nötige Dichtigkeit des Serums und des Plasmas ist sowie die unumgängliche zeitliche Bedingung, die auf die Bazillen ihren Einfluss ausüben, welche beiden Bedingungen beim Experimente nicht vernachlässigt werden dürfen.

3) Sowohl durch die Slide Cell Culture als auch durch die Tiefenkultur lässt sich die Neigung erkennen, dass das Bazillenwachstum umso lebhafter wird, je mehr die Tage der Kultur sich mehren. Bei der Slide Cell Culture ist es ungefähr am 5. bzw. 7. Tage, bei der Tiefenkultur am 10. bzw. 20. Tage, wo ihr Unterschied von den Kontrollen deutlich zu

erkennen ist. Dies lässt auch vermuten, dass Vollblut, Serum und Plasma während der verlängerten Tage der Kultur eine gewisse Aenderung erfahren, sodass sich die in ihnen befindliche hemmende Wirkung allmählich abschwächt und die Wachstumsfähigkeit der Bazillen dann plötzlich lebhaft wird, bis Vollblut, Serum und Plasma, die bis dahin hemmend gewirkt hatten, sich in den Bazillenwachstum fördernden neuen Nährboden umwandeln.

Siese Tatsache lässt ferner die Vermutung zu, dass diese entwicklungshemmende Wirkung keine bakterizide, sondern vielmehr eine bakteriostatische Wirkung ist.

V. Zusammenfassung.

1) Man erkennt durch die Slide Cell Culture im Vollblut von Ratte, Hund, Pferd, Kaninchen usw. eine entwicklungsehmmende Wirkung auf Tuberkelbazillen, obwohl diese Wirkung, abgesehen von der Ratte, im allgemeinen schwach ist.

2) In Phosphat-Natr. glutaminicum-Glycerin-Nährflüssigkeit, die 20% Serum od 50% Plasma des Hundes sowie des Pferdes enthält, bemerkt man ein deutliches Vorhandensein der entwicklungshemmenden Wirkung, wenn man Tuberkelbazillen in der Tiefe der Flüssigkeit über 10 Tage lang kultiviert.

3) Diese Wirkung ist sowohl in der Slide Cell Culture als auch bei der Tiefenkultur im Anfangsstadium der Kultur besonders deutlich. Deshalb kann man sagen, dass die entwicklungshemmende Wirkung des Serums oder des Plasmas auf Tuberkelbazillen keine bakterizide, sondern vielmehr eine bakteriostatische Wirkung ist.

Diese Forschung wurde durch Überlassung von Geldmitteln des Unterrichtsministeriums fur wissenschaftliche Forschung ausgeführt, wofür herzlich gedankt sei. . . . Prof. Dr. T. Kumagai und Prof. Dr. T. Ebina.

Literatur.

1) A. E. Wright; The Lancet, 1924, **206**, 218.
2) Yabusita; Tokyo-Igaku-Zassi, 1933, Nr. 2858, 2832.
3) Miwa; Saikingaku-Zassi, 1940, Nr. 529, 152.
4) Sato; Zikkenigaku, 1926, **10**, 871.
5) Ito; Kekkaku, 1930, **8**, 291.
6) Horai; Kekkaku, 1934, **17**, 621
7) Uesaka; Kekkaku, 1936, **14**, 578.
8) Kirchner; Zbl. Bakt. Inf. Orig., 1932, **124**, 403.
9) Miyasaka; Tohoku-Igaku-Zassi, 1940, **26**, 143.
10) Meissner; Zbl. Bakt. Orig., 1928, **106**, 210.
11) Kuwahar5; Kekkaku, 1941, **19**, 530.

The Tohoku Journal of Experimental Medicine, Vol. 50, Nos. 3 & 4, 1949.

Our Experiences upon Chemotherapy of Lung Tuberculosis with „ Cepharanthin".

By

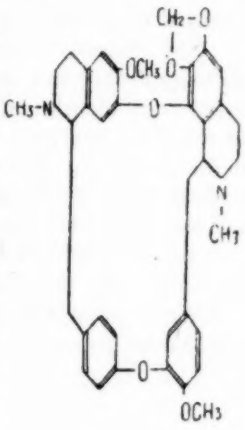
Tosiaki Ebina and Iwao Kanno
(海老名俊明)　(菅野巌)
(Institute of Tuberculosis and Leprosy (Kosankinbyo Kenkyusho), Tohoku Imperial University, Sendai.)

and

Kaoru Yamasaki.
(山崎馨)
(Tuberculosis Sanatorium " Kofuen," Yamagata.)

(Received for publication, July 10, 1946)

With the discovery of sulfanilamide, the study of the chemotheraphy of tuberculosis has been stimulated in this country. Several chemotherapeutic agents have been reported as beneficial, but " Cepharanthin " is the center of most interest and discussion.



Hasegawa and his coworkers[1] have studied the influences of Biscoclaurin-Alkaloid upon experimental tuberculosis of guinea-pigs and found that the total Alkaloid of a plant named " Stephania Cepharantha," which prospers in Formosa, impedes tubercle formation. According to the chemical study of Kondo[2] Cepharanthin is a light yellow powder, solble in organic solvents except petroleum ether, it's m.p. is 140–145°, $[\alpha]^{21}=+204°$, the molecular weight (606, 690). empirical formula C_{37} H_{38} N_2 O_6 and the structural formula as shown in the Figure Komiya, Kaneko and others[3] have reported that " Cepharanthin " has a beneficial effect on human pulmonary tuberculosis.

According to Hashimoto and Akasaka[4] this medicine is useful in treating tuberculosis of the skin and of the urogenital tract and according to Ofuji[5] for the tuberculosis of the neck. Iizumi[6] and Wataai[7] maintained that " Cepharantin " is also of value in the prophylaxis of tuberculosis. The dosage, employed by different authors,

varies. But all the authors write in their special volume "Chemotherapy of Tuberculosis" altogether as follows.

"As the applyingsmethods the oral and intravenous ways are recommanded. When the illness is progressive, the dosage of Cepharanthin must be 0,1–0,3 mg but when the sickness is light, it can be 1,0 mg. When the fever is high, it must be 0,1 mg, although the process is light. The medicine is given once in a week intravenously and 6 times a week orally. Sometimes it can be given orally once every two or three days. In short, when the fever, the acceleration of the sedimentation velocity of red blood cells and subjective hardships are in a high degree, it should be given orally 0.05–0.1 mg every two days. When the illness shows an aggravating tendency, then the dose must be reduced or the drug must be stopped. When the sickness becomes better, then one can increase the dosage to more than 0.2 mg." From this explanation of the applying method, we can judge immediately that Cepharanthin is a very dangerous drug because it can do harm to the patient, when it is overdosed a little.

We have attempted to evaluate the use of this drug in pulmonary tuberculosis. The patients, that we have treated with it, were patients of our hospitals and were observed for as long as one or two years. A group consisted in 8 relatively mild and 7 relatively severe cases. B group consisted in 6 mild, 7 moderate and 6 severe cases.

The patients were examined physically every day and roentgenphotographed every month. The erythrocyte sedimentation rate was tested every month and the sputum every week. In regard to the dosage, we were very careful. We followed, in general, the explanatory statement of the medicine. For the A group we raised the dosage from 0.01 mg gradually and slowly until 0.05 mg pro dies. It was given orally every day except Sunday for about 5 months. For the B group we have given light cases orally 0.05 mg, moderate cases 0.025 mg and severe cases 0.01 mg every other week for half a year.

When patients showed any symptom of aggravation, then the remedy was at once given up.

Results of the therapy.

X-Ray findings, erythrocyte sedimentation rates, body weights, tubercle bacilli in sputum, sputumnumber and body temperature are charted in tables (I-V)

The results shown may be summarized as follows.

A Group.

The I. Division Mild cases.

Total cases 8

		case
1)	X-Ray Findings aggravated	1
2)	Erythrocyte sedimentation rate increased	5
3)	Body weight decreased	5
4)	Tubercle bacilli in sputum increased	4
5)	Fever aggravated	4
6)	Sputum distinctly increased	3
7)	Hemoptysis	1
8)	General health conditions improved	0

The II Division. Relatively severe cases.

	Total cases	7
1)	X-Ray Findings aggravated	2
2)	Sedimentation rate increassed	3
3)	Body weight distinctly decreased	3
4)	Tubercle bacilli increased	4
5)	Fever agravated	3
6)	Sputum increased	2
7)	Hemoptysis	1
8)	General health conditions improved	0

B Group.

The I Division. Mild cases.

	Total cases	6
		case
1)	Some change on X-ray Findings	0
2)	Sedimentation rate increased	0
3)	Body weight increased	2
4)	Body temperature raised	0
5)	Physical symptoms aggravated	0
6)	General health condition changed	0

The II Division. Moderately severe cases

	Total cases	7
1)	X-Ray Findings aggravated	3
2)	Sedimentation rate increased	4
3)	Body weight decreased	4
4)	Tubercle bacilli in sputum increased	4
5)	Fever aggravated	4
6)	Bloody sputum or hemoptysis	2
7)	Physical symptoms aggravated	4
8)	General health conditions improved	0
9)	Dead	2

The III Division. Severe cases

	Total cases	6
1)	X-Ray Findings aggravated	3
2)	Sedimentation rate increased	3
3)	Tubercle bacilli in sputum increased	2
4)	Fever aggravated	5
5)	Bloody sputum or hemoptysis	3
6)	Physical symptoms aggravated	3
7)	General health conditions improved	0

TABLE

(1) *Influence of cepharanthin upon the progress of*

Dose: 19/VI–16/VII 1944 . . 0,01 mg, 17/VII–30/VII 1944 . . 0,02 mg, 31/VIII–25/

Nr.	Name, age and gender	X-ray finding		Sedimentatioe velocity of red blood cells. Fall in mm in 1 hour	
		*B. C. Th.	Within 4 months *A. C. Th.	B. C. Th.	A. C. Th within *4M
1	M.M. 29 ♂	Indurated partially calcinated shadow in left upper lobe.	No change	4	hardly any change
2	S.A. 41 ♂	Multiple indurated and calcific foci in all lobes of the both lungs.	No change	34	40–56
3	O.S. 30 ♂	Indurated shadow in both upper lung fields	No change	10	hardly any change
4	K. J. 23 ♂	Indurated partially calcinated shadow in both upper lung fields.	shadow increased	10	17–22
5	S.K. 23 ♀	Indurated shadow in left upper lung field	After 3 months shadow slightly absorbed	4	14–26
6	A.M. 20 ♀	Enlargement in the right mediastinal shadow	New shadow in right upper lung field	29	40–65
7	N.Y. 38 ♀	Indurated shadow in left upper lung field.	No change	35	hardly any change
8	S.I. 30 ♀	Multiple productive foci in both lungs: cavities in both upper lung fields.	After 4 months shadow absorbed a little	14	6–25

*B. C. Th. . . . Before the cepharanthin therapy.
A. C. Th. . . . After the beginning of the cepharanthin therapy.
M. . . . month.

8) Dead 3

The summary of the A group shows that light cases as well as relatively severe cases could not have the recovery of general health condition, but on the contrary about half of them showed aggravating symptoms such as the acceleration of sedimentation velocity of red blood cells, the increase of tubercle bacilli in sputum and aggravation of fever: two cases had hemoptysis. From the short summary of the B group it is clear, that very light cases were not so much influenced by Cepharanthin as moderately severe and severe cases, which were mostly harmed by this remedy. About

I. A Group.

tuberculosis of the lung (relatively light cases)

VIII 1944 . . 0,03 mg. 26/VIII–15/X 1944 . . 0,04mg, 16/X –12/XI 1944 . . 0,05mg.

Body weight (Kg)		Tubercle-bacilli in sputum. C: Culture GF: Gaffky				Sputum count		Body temperature (°C)	
B. C. Th.	A. C. Th. within 4 M.	B. C. Th.	A. C. Th. IM.	A. C. Th. II M.	A. C. Th. IIIM.	B. C. Th.	A. C. Th. within 4 M.	B. C. Th.	A. C. Th. within 4 M.
56,4	III–IV Mon. 53,3–54,6	C. (−)	C. (+)	C. (+)	C. (++)	5 1 20	III. M. 20–25	37,1	II–III M. 37,3
70,8	hardly any change	C. (+)	C. (−)	C. (+)	C. (−)	6 1 7	III. M. 10	36,8	No change
69,0	67,2–68.0	GF I	GF I	C. (++)		10	No change	36,8	hardly any change
55,3	hardly any change	C. (+)	C. (++)	C. (++)	C. (+)	2	No change	37,0	I–II M. 37,1
43,5	41,0–40,5	C. (+)	C. (+)	C. (+)	C. (−)	5	No change	37,0	I–II M. 37,3–37,5
50,4	III–IV Mon. 48,9–49,0	C. (−)	C. (−)	C. (−)		4	I–II M. 7–15 IV. M. 8–10	37,0	I–II M. 37,2 III–IV M. 37,1
52,0	hardly any change	GF II	GF I	GF II	GF I	4 1 5	No change	37,1	hardly any change
53,5	49,6–52,3	GF V	GF X	GF IX	GF IX	25 1 30	II–IV M. 35–40	37,0	I–II M. 37,2–37,3

Remarks: Nr. 1 From 8/IX 1944 walk permitted, Nr. 4 29/IX 1944 Discharged, Nr 6 4/VIII 1944 Stop Th., Nr. 8 9/X–13/X 1944 Blood sputum.

half of them had an acceleration of erythrocyte sedimentation rate, decrease of body weight, increase of tubercle bacilli in sputum, increase of râles, rise of fever and often hemoptysis. Four of moderately severe and severe cases died.

Our results in these experiments lead us to conclude, that one can apply only small doses of this medicine to very light not progressive cases without aggravation of disease, but we can not use it, when the disease is active or progressive, although the doses are very small as 0.01 or 0.025 mg.

Okuda[8] reported in the committee on chemotherapeutic research of tuberculosis, Feb. 9. 1945, Tokio, that he gave 26 patients Cepharanthin

TABLE

(2) *Influence of cepharanthin upon the progress of tuberculosis*

Dose: 17/VII–30/VII 1944 . . 0,01 mg, 31/VII–25/VIII 1944 . . 0,02 mg, 26/VIII–15/X

Nr.	Name, age and gender	X-ray finding		Sedimentation velocity of red blood cells	
		B. C. Th.	Within 3 months A. C. Th.	B. C. Th.	A. C. Th. within 3 M.
1	I.S. 22 ♂	Exudative shadow in both lungs. left Pyothorax.	No change	50	91–113
2	T.N. 22 ♂	Productive shadows in both upper lung fields. Cavities in the both lungs.	No change	20	hardly any change
3	A.S. 21 ♂	Exudative shadows and cavities in the right upper lung field.	shadow slightly resorbed	9	13–19
4	A.T. 26 ♂	Exudative shadows in the left lung field. A little infiltration in the right apex.	shadow in the left lung increased	22	hardly any change
5	H.K. 21 ♂	Exudative shadows in the right lung. Cavities in the upper lung field.	After 1 month an infiltration in the left upper lung field and after 2 months more distinct	78	hardly any change
6	Y.M. 30 ♂	Exudative partially productive shadows in both lungs. cavities in both upper lung fields.	No change	42	hardly any change
7	M.T. 33 ♂	Exudative shadows in both lungs. cavities in both upper lung fields.	After 3 months exudative pleuritis in the left lung field.	29	48–94

Remarks: Nr. 5 the medication discontinued on 20/VIII 1944.
Nr. 6 2/IX–9/IX 1944, hemoptoe,

0.01–0.2mg orally every two days during 8 months but he could not get better results than comparison.

In regard to the mechanism of this medicine we know hardly anything Hasegawa[9] writes in his book that it is questionable whether the therapy with Cepharanthin is called a chemotherapy but it may come within the purview of the definition of the chemotherapy and he writes farther, that is may be justified at the present, when the mechanism of its effect is not quite known to us. Recently in the committee on chemotherapeutic research of tuberculosis Feb. 9, 1945. Tokio he counted up as affecting mechanism 3 factors, namely bacteriostatic effect upon tubercle bacilli, antiallergic and stimulating effect upon the reticuloendothelum.

II. A Group.

of the lung (relatively serious cases)

1944 . . 0,03 mg, 16/X–12/XI 1944 . . 0,05 mg.

Body weight (Kg)		Tubercle bacilli in sputum				Sputum count		Body temperature (°C)	
B. C. Th.	A. C. Th. within 3 M.	B. C. Th.	A. C. Th. I M.	A. C. Th. II M.	A. C. Th. III M.	B. C. Th.	A. C. Th. within 3 M.	B. C. Th.	A. C. Th. within 3 M.
54,2	hardly any change	C (+)				1	No change	36,8	hardly any change
58,0	hardly any change	GF. I	GF. X	GF. II	GF. (−)	20	No change	36,7	No change
56,6	55,5–55,8	GF. V	GF. VII	GF. I	GF. (−)	7	No change	37,2	hardly any change
57,8	II–III M. 55,2–550	GE. VIII	GF. II	GF. VIII	GF. VIII	5	hardly any change	36,9	After 2–3 M. 37,0–37,8
59,7	II–III M. 56,2–56,9	GF. VIII	GF. X	GF. VIII	GF. VIII	6	After one month sputum increased (10)	37,3	21/VIII–5/IX 1944 37,5–38,5 20/IX–7/X 1944 37,4–38,5
44,0	hardly any change	GF. VIII	GF. VIII	GF. VIII	GF. X	25	hardly any change	37,2	hardly any change
48,6	II–III M. 45,4–45,8	GF. I	GF. I	GF. II	GF. III	10 1 15	After 1–2 mos. 20–30 After 3 mos. 30–40	37,0	28/IX–28/X 1944 37,5–38,0

Nr. 7 20/VII–10/VIII l. artificial pneumothorax three times.

TABLE III. B Group.

(1) *Influence of cepharanthin upon the progress of tuberculosis of the lung. Light cases (closed tuberculosis)*

Dose: Cepharanthin given daily 0,05 mg every other week during 6 months.

Nr.	Name, age and gender	Diagnosis, X-ray finding	Sedimentation velocity of red blood cells		Body weight (kg)		Tubercle bacilli in sputum							Body temperature (°C)		Physical examination in the lungs	
			B. C. Th.	A. C. Th. within 6 mos.	B. C. Th.	A. C. Th. within 6 mos.	B. C. Th.	A. C. Th.						B. C. Th.	A.C. Th. within 6 mos.	B. C. Th.	A. C. Th. within 6 mos.
								I M.	II M.	III M.	IV M.	V M.	VI M.				
1	Y. I. 35 ♂	Tbc. pulm. sin. a cirrhosic shadow in l. Apex.	5	hardly any change	57,0	hardly any change	GF. (−)	GF. (−)	GF. (−)	GF. (−)	GF. (−)	GF. (−)	GF. (−)	36,8	After 3 mos. 37,0	no dullness no râle	no change
2	S. W. 25 ♂	Tbc. pulm. duplex a cirrhosic shadow in r. lower lobe.	2	hardly any change	50,0	hardly any change	GF. (−)	GF. (−)	GF. (−)	GF. (−)	GF. (−)	GF. (−)	GF. (−)	36,5	no chagge	no râle	no change
3	H. H. 22 ♂	Tbc. pulm. duplex a cirrhosic shadow in both lungs.	30	hardly any change	48,8	II–VI. M. 49,4–53,0	GF. (−)	GF. (−)	GF. (−)	GF. (−)	GF. (−)	GF. (−)	GF. (−)	37,2	hardly any change	R. below back dullness no râle	no change
4	A. E. 17 ♂	Apex tuberculosis â cirrnosic shadow in both apexes.	3	hardly any change	49,0	hardly any change	GF. (−)	GF. (−)	GF. (−)	GF. (−)	GF. (−)	GF. (−)		36,8	No change	no dullness no râle	no change
5	T. K. 21 ♂	Primary infection a few shadows in r. hilus.	7	hardly any change	51,0	II–VI. M. 53,0–54,3	GF. (−)	GF. (−)	GF. (−)	GF. (−)	GF. (−)	GF. (−)	GF. (−)	36,8	No change	no dullness no râle	no change
6	M. K. 18 ♂	Primary infection no shadow.	8	hardly any change	50,0	hardly any change	GF. (−)	GF. (−)	no sputum	〃	〃	〃	〃	36,7	No change	no dullness no râle	no change

Remarks: Nr. 1, Nr. 2, Nr. 4, Nr. 6, After the admission, the progress favourable.

In the same committe Toda[10] reported his animal experiments with Cepharanthin as follows:

1) Cepharanthin can not work upon tubercle bacilli bactericidally or bacteriostatically in animal body, on the contrary it promotes sometimes growth of bacilli when the medicine is given too much.

2) Cepharanthin has no bactericidal effect also in vitro.

3) Cepharanthin has to some extent an impeding effect of ulcer formation or promoting effect of the healing.

4) Cepharanthin has characters of a stimulating agent and is not called a specific chemotherapeutic agent.

According to Oka and Yanagizawa[11] it is not affirmed that Cepharanthin has a specific therapeutic effect upon the experimental tuberculosis in guinea pigs in any times after the infection, on the contrary tuberculous processes some times can be accelerated by it, when it is given continuously for such a long time as 23 weeks.

From their results we can suppose that Cepharanthin may be a remedy which acts as stimulating agents against tuberculosis, therefore it can often benefit his health when the patient suffers from chronic not progressive tuberculosis, but it can more damage the health, when the disease is exudative and progressive or the remedy is given too much.

Conclusions.

Cepharanthin was given 34 tuberculous patients 0.01–0.05mg pro dies orally every day or every other week for about 6 months and we got the following results.

Mild cases with no exudative symptoms were not appreciably influenced by Cepharanthin but moderately severe and very severe cases with exudative processes were harmfully influenced. Most of them showed aggravated X-Ray Findings, increased sedimentation velocity of red blood cells, increased tubercle bacilli in sputum and raised body temperature. None showed improvement in general health and no severe case was saved from death.

Literatur

1) Hasegawa: Tokyo Ijishinshi, 1935, **2956**, 2939.
Kekkaku, 1936, **14**, 383.
" 1938, **16**, 731.
" 1939 **17**, 616.
" "Kekkaku no Kagakuryoho ni kansuru Kenkyu," Tokyo, 1942, 9.

2) Kondo: "Kekkaku no Kagakuryoho ni kansuru Kenkyu," 1942, 169.

3) Komiya, Kaneko and others: The same book, 410, 423, 487.

4) Hashimoto and Akasaka: The same book, 569.

5) Ofuji: The same book, 854.
6) Iizumi: The same book, 597.
7) Wataai: The same book 906.
8) Okuda: not yet printed.

TABLE IV.

(2) *Influence of cepharanthin upon the progress of tuberculosis*

Dose: Cepharanthin given daily 0,025 mg

Nr.	name, age and gender	Diagnosis, X-ray finding	Sedimentation velocity of red blood cells		Body weight (Kg)		Tubercle bacilli in sputum				
			B. C. Th.	A. C. Th. within 6 M.	B. C. Th.	A. B. Th. within 6 M.	B.C. Th.	A. C. Th.			
								I M.	IIM.	III M.	IV M.	V M.	VI M.
1	S. H. 50 ♂	Tbc. pulm. duplex large cavernous shadows in both lungs	32	15–25	63.6	hardly any change	GF. V	GF. V	GF. VII	GF. V			
2	T. A. 22 ♀	Tbc. pulm. sin. wide cirrhosic shadows in l. lung	12	hardly any change	53,5	I–V. M. 50,0–52,9	GF. (–)	GF. (–)	GF. (–)	GF. (–)	GF. (–)	GF. (–)	GF. (–)
3	K. O. 25 ♀	Tbc. pulm. duplex with larynxtbc. Exudative shadows in all fields	35	54–60	48,9	I.–II. M. 46,6–46,8	GF. V	GF. V	GF. V			GF. VII	GF. VII
4	K. S. ♂	Tbc. pulm. duplex with Epididymitis tbc. Giant cavities in both lungs	76	hardly any change	54,2	IV.–VI. 51,9–52,3	GF. VII	GF. X	GF. X	GF. X	GF. VII	GF. VII	GF. X
5	O. S. 28 ♂	Tbc. pulm. duplex wide productive shadows in both lungs	33	38–46	52,0	V. M. 48,8	GF. VII	GF. VII	GF. VII	GF. VII.		GF. VII	GF. VII
6	R. M. 22 ♂	Tbc. pulm. sin. Productive shadows in upper lobe	19	III.–V. M. 23–36	48,8	49,8–54,4	GF. (–)	GF. (–)	GF. III	GF. (–)	GF. (–)	GF. (–)	GF. (–)
7	T. E. 20 ♀	Haematogenous dissemination with renal tuberculosis	105	122–127	35,8		GF. (–)	GF. (–)	GF. (–)	GF. (–)	GF. (–)	GF. (–)	GF. (–)

9) Hasegawa: not yet printed.
10) Toda: not yet printed.
11) Oka and Yanagizawa: not yet printed.

B. Group.

of lung (Middle serious tuberculosis)

every other week during 6 months.

Body temperature (°C) B. C. Th.	Body temperature (°C) A. C. Th. within 6 mos.	Physical examination in the lung B. C. Th.	A. C. Th. I M.	II M.	III M.	IV M.	V M.	VI M.	Remarks.
36,7	no change	R. upper back râle	"	no râle no dullness	"	"	no râle no dullness		Oct. 17, 1944 discharged. The progress favourable.
36,8	no change	no râle	"	"	"	"	"	"	After the admission, the progress favourable.
37,0	37,2–38,0	R. consonating râle	"	"	Râle cough and sputum increased	"	"	"	Since Sep. 14, 1944 no "Ceph." was given because of the aggravating of general condition. Jan. 14, 1945 died.
36,7	37,2–37,5	R. upper front bubbling râles	"	"	Râles increased	"	Râles decreased	"	On account of the increasing râles "Ceph." given up since Sep. 27, 1944. Hemoptoe Oct. 13, Jan. 8, 1945 Epididymitis discovered.
36,8	37,5–37,7	L. râle scanty	Râle increased	Râles increased "ceph." adm. stopped	"	L. râles everywhere	"	"	Fever, râles increased the general state aggravated. Sep. 10, bloody sputum. X-Ray finding also aggravated. "Ceph." given since Sep. 21.
36,8	no change	L. râles scanty	"	Râles increased	Râles	no "Ceph." given since 1. nov.			On account of increasing râles given no "Ceph." since Nov. 1. Thereafter the general state again improved.
37,0	37,2–37,5	L. râle	"	"	"		"	"	The general states aggravated. No "Ceph." given since Aug. 24. Dec. 21, 1944 died.

TABLE V.

(3) *Influence of cepharanthin upon the progress of tuberculosis of*

Dose: Cepharanthin given daily 0,01 mg

Nr.	Name, age and gender	Diagnosis X-ray finding	Sedimentation velocity of red blood cells		Body weight (Kg)		Tubercle bacilli in sputum						
			B. C. Th.	A. C. Te. within 6 mos.	B. C. Th.	A. C. Th. within 6 M.	B. C. Th.	A. C. Th. I M.	A. C. Th. II M.	A. C. Th. III M.	A. C. Th. IV M.	A. C. Th. V M.	A. C. Th. VI M.
1	S. K. 26 ♂	Tbc. pulm. duplex Exudative shadows in both lungs.	64	hardly any change	58,8	hardly any change	GF. VIII	GF. VIII	GF. VIII	GF. VIII	GF. VIII		
2	S. S. 34 ♂	Tbc. pulm. duplex with Laryngitis tbc. Exudative productive shadows in both lungs.	48	I M. 61	50,5	53,4–56.9	GF. VIII	GF. VIII	GF. VIII	GF. VIII	GF. VI	GF. VIII	GF. VIII
3	S. S. 28 ♂	Tbc. pulm. duplex wide exudative shadows in both lungs.	65	I.–V. M. 69–74	48,9	hardly any change	GF. VII	GF. X	GF. X	GF. X	GF. X	GF. X	GF. X
4	H. A. 20 ♂	Tbc. pulm. duplex Exudative cavernous shadows in both lungs.	48	II.–IV. M. 53–105	43,0	hardly any change	GF. VIII	GF. VIII	GF. VIII	GF. VIII	GF. VIII	GF. VIII	GF. VIII
5	T. N. 19 ♂	Tbc. pulm. duplex Enterocolitis tbc. Exudative shadows in both lungs.	110	hardly any change	59,0		GF. VIII	GF. VIII	GF. VIII	GF. VIII			
6	X. T. 24 ♀	Tbc. pulm. duplex Exudative shadows in both lungs.	117	hardly any change	37,4		GF. VIII	GF. VII	GF. VII	GF. X	GF. X	GF. X	GF. X

B. Group.

the lung. (*serious cases*)

every other week during 6 months.

Body temperature (°C)		Physical examination in lungs.							Remarks
B. C. Th.	A.C. Th. within 6 M.	B. C. Th.	A. C. Th. I M.	II M.	III M.	IV M.	V M.	VI M.	
36,8	37,0–38,0 –39,0	L. Râles and dullness	Râles a little decreased		Râles increased but no grievance	"	Hemoptoe râles increased difficult breathing		Nov. 17, 1944 Hemoptoe 200cc. Nov. 20, Hemoptoe 180cc. Fever 38–39,5 °C. Caseous peumonie. Nov. 25, died.
36,9	Almost no change	R. behind dullness no râles	L. behind râles R. no râles	"	"	"	"	"	The progress favourable
36,8	37,2–37,5	R. no dullness. no râles L. râles	L. râles increased	"	L. râles decreased	"			After the beginning of this therapy, X–ray finding aggravated. Hemoptoe Aug. 11, 1944. Administration of the drug stopped. Thereafter the progress favourable.
36,8	37,5–38,0	Both sides râles difficult breathing	"	Both sides râles increased difficult breathing become more severe		Râles everywhere heard	"		On account of fever and emaciation no "Ceph," given since Sept. 11, 1944. Dec. 26, died.
37,2	37,2–37,4	R. scanty râles L. everywhere râles	"	"	Difficult breathing				Because of aggravated general state no "Ceph." given since Sept. 20., Oct. 18, 1944 died.
36,8	36,8–37,3	R. everywhere râles	R. everywhere râles cough a. sputum increased		R. everywhere râles				Because of Hemoptoe no "Ceph." given since Nov. 11, 1944.

The Tohoku Journal of Experimental Medicine, Vol. 50, Nos. 3 & 4, 1949.

Biochemical Studies on Carbohydrates.

XCVII. On the A Group-Specific Carbohydrates from Stomach Mucus of Bull and of Horse.

By

Hirosi Tiba.

(千葉　博)

(From the Medico-Chemical Institute, Tohoku Imperial University, Sendai. Director: Prof. H. Masamune.)

(Received for publication, March 30, 1946)

Sinokawa[1] demonstrated unobjectionably that the group carbohydrate of mucus-mucin of hog stomachs can be separated in a pure state by fractionating the alkaline scission products of the mucus itself. Based on this proof, we began to prepare this carbohydrate from animals of different species in order to compare their chemical composition, physical properties and biological activity. The present account deals with those of bull and horse. The carbohydrate from the fourth stomach of bulls was confirmed to be identical with the one from hog stomachs. But the horse carbohydate differed from them, galactose markedly exceeding hexosamine (the hog and bull substances contain these sugar components in equimolecular ratio) and its A activity being extremely less. Moreover it rotated D-light more to the left. The exact nature of the amino sugar in it could not be established owing to the lack of material, but it may be most probably assumed to be glucosamine as is hexosamine which constitutes most of the A group substances from animal sources hitherto studied. What structure is responsible for the group specific action is a problem soluble only by a persevering effort, but at least in the horse carbohydrate the enormous excess of galactose over hexosamine appears to be a detriment to this effect.

Incidentally it should be noted that the bull and horse carbohydrates proved non-poisonous in 10 mgm. dosis per 10 gm. body wieght when injected intraperitoneally to a mouse, and also that the bull carbohydrate has been found scisille by the snail enzyme[2].

EXPERIMENTAL.

Assay for Group Specificness of Stomach Mucus.

The fresh mucus from each stomach was tested for its group specifity

in advance to the use for separating the carbohydrate. The procedure of Sinokawa[1] to examine the mucin was followed, replacing the dry mucin with the same weight (10 mgm.) of wet mucus. The bull stomach mucus showed A activity up to 100–1000 times dilution of the original suspensions (1%), but in cases of the horse mucus, only the original suspensions inhibited the isoagglutionation of A erythrocytes and the 10 times diluted did not anymore. All the materials examined did not display B activity but A activity.

Separation of the Group Carbohydrates and Chemical Qualitative Properties of the Preparations.

The preparation method of Kobayasi[3] was modified by omitting kaolin treatment and combining fractionation with glycol. A run started with the bull stomach (fourth) mucus will be described in full.

The fresh mucus was scraped off the cleansed inner surface of 28 bull stomachs and after the mucus from each of the stomachs had been proved A specific, all the lumps were united. The batch amounting to 620 gm. was stood with 2 volumes of 1.5 n. NaOH solution in air (15–20°C) for 24 hours. The solution was then neutralized under cooling in ice water with small portions of glacial acetic acid and acidified further to pH 5.0 with 30% acetic acid. The appearing precipitates were separated off by centrifugalization and the supernatant liquid dialyzed (urine bladder) against running water for 3 days. The dialyzed liquid was again acidulated and the protein fragment newly thrown down having been centrifuged off, evaporated to a syrupy consistency under reduced pressure. Absolute alcohol was added and the dehydrated residue washed with absolute alcohol twice and with ether and dried by means of a jet pump. In the second stage, the powder obtained (6 gm.) was kneaded at first with a small quantity of ethylene glycol and followingly the solvent was increased to 150 cc. Thoroughly agitated and repeatedly centrifuged. The supernatant transparent brown liquid was precipitated with absolute alcohol and the precipitates washed and dried as above. Yield, 3 gm. In the third stage the substance was taken up in 4 times the weight of water, the portion remaining undissolved removed by prolonged centrifugation and 15 volumes of glacial acetic acid (96%) added, whereupon precipitation occurred. Without separating off of the precipitates the mixture was shaken as such on an apparatus for 3 days and subsequently centrifuzed. The centrifugate was treated with changes of alcohol, until all acetic acid was washed out, then with ether and dried. Crop, 1.38 gm.

Since positive biuret reaction was still given, fractionation with glycol with all the following processes were repeated twice more, when no biuret-giving substance was detected. 0.3 gm. of non-coloured powder was

yielded. This final product (Prep. I) was perfectly water-soluble. By Molisch reagents developed bright violet colour (not dirty red). Phosphorus (ammonium molybdate test) and hexuronic acid (Neuberg and Saneyoshi naphthoresorcinol test) were absent, but hydrolysable sulfur present. Ninhydrin reaction was scarcely shown. Arginine (Sukaguchi) was present in trace, but histidine was not at all. Osaki and Turumi test: indirect procedure, postive; direct procedure, almost negative.

The corresponding carbohydrate was prepared from horse stomach-mucus in a similar manner but in the initial treatment with glacial acetic acid almost all went into solution so that I was compelled to condense the solution at a low temperature to a small volume and again add glacial acetic acid. 40 mgm. were obtined from 600 gm. mucus collected from 17 stomachs, i.e., the yield was much less than from bull mucus. The substance gave a clear solution in water. Biuret, ninhydrin, ammonium molybdate (for phosphorus), Ehrlich dizao (for histidine) and naphthoresorcinol (for hexuronic acid) tests resulted in negative. Arginine (Sakaguchi) was detected, though little. Dimethylaminobenzaldehyde gave red colour after the alkaline solution of the substance had been heated, but did not when this preliminary treatment was omitted. The presence of hydrolysable sulfur ($BaSO_4$ method) revealed itself.

Quantitative-Analytical Figures and Optical Rotatory Power of the Carbohydrates.

Analysis of the preparations by the methods, which had been hitherto employed by us, gave values in Table I.

TABLE I.

Analysis	In per cent		In equivalents per equivalent weight	
	Bull carbohydrate (Prep. I)	Horse carbohydrate	Bull carbohydrate (Prep. I)	Horse carbohydrate
N*	5.12	2.32	1.49	2.34
Hexosamine	43.8	12.3	1.0	1.0
Acetyl	10.46		0.99	
Galactose	44.1	57.8	1.00	4.67
Indole r.	43.8	57.5		
Orcinol r.	44.2	58.3		
Diphenylamine I r.	44.2	57.5		
Hydrolysable sulfur	0.94		0.12	
Ash†	2.5			

* Micro-Kjeldahl method. † Micro analysis

Optical rotation in water.

Bull carbohydrate:

$$[\alpha]_D^{22} = \frac{-0.19 \times 3.0533}{0.0208 \times 0.998 \times 1} = -28.7°.$$

Horse carbohydrate:

$$[\alpha]_D^{20} = \frac{-0.20 \times 2.9905}{0.0167 \times 0.9999 \times 1} = -35.8°.$$

The values of the bull polysaccharide thus coincided nearly with those of the polysaccharide from hog stomach mucus (cf. Sinokawa[1]). With respect to hydrolysable sulfur in this polysaccharide Sinokawa asserted that it is contained in the molecular ratio to glucosamine of about 0.13: 1.0 which is materially less than that found by Kobayasi[3][4]. I adapted the present method of preparation to crude mucus mucin of hog stomachs which had been prepared by Kobayasi[4] and substantiated Sinokawa's claim.

The hog carbohydrate prepared by me was similar qualitatively as well as quantitatively as Sinokawa's, namely:

Biuret, naphthoresorcinol and Ehrlich diazo (for histidine) tests, all negative. Ninhydrin, almost negative. Molisch, intensively positive (violet coloration). Sakaguchi test (for arginine), faintly positive. Indirect Osaki and Turumi test, positive; the direct, very faint (coloration occurred between the 7th and 10th minutes of heating). Nitrogen 5.46%; glucosamine, 43.18%; galactose, 42.8%; hydrolysable sulfur, 0.97% (0.126 equivalent of glucosamine); ash, 3.85%. Measurement of the group specific activity (method vide below; serum titre: A serum 160, B serum 80) gave:

Dilution of substance / Group of serum	Orig. sol. (1%)	$\times 10$	$\times 10^2$	$\times 10^3$	$\times 10^4$	$\times 10^5$
A	++	++	++	++	++	++
B	−	−	−	−	−	+

Furthermore the hexosamine and hexose in the bull carbohydrate was demonstrated to be glucosamine and galactose as below which is regarded as an additional argument for the identity of these polysaccharides.

On the contrary, the horse carbohydrate can be stated definitely divergent from them even when viewed merely from the molecular ratio between hexosamine and hexose and from optical property, although shortage of material did not allow a detailed study.

Identification of Glucosamine and Galactose in the Bull Carbohydrate.

200 mgm. of another specimen of the bull carbohydrate obtained likely as Prep. I were submitted to hydrolysis and following fractionation according to Satoh et al[5].

The hexose fraction removed from Ba and Hg was neutralized with a dilute alkali (NaOH), condensed under reduced pressure to 5 cc. and a methylphenylhydrazone was prepared after Lüdtke[6]. The crude product amounted to 48 mgm. Recrystallized twice from 95% alcohol, it (thick needles; yield, 37 mgm.) melted at 190–190.5° and no depression of melting point occurred, when it was mixed with methylphenylhydrazone from galactose. N (micro Dumas) 9.93% (calc. for $C_{13}H_{20}O_5N_2$, 9.85%).

From the hexosamine fraction were obtained 60 mgm. of crude glucoaminepentabenzoate after Satoh et al. The pure product (32 mgm.) after repeated (3 times) crystallization melted at 216°C and showed in pyridine $[\alpha]_D^2 = \frac{0.53 \times 1.2933}{0.0155 \times 0.978 \times 1} = +45.2°$. N (micro Kjeldahl), 1.98% (calc., 2.00%).

Specific Anti-Isoagglutinative Activity of the Carbohydrates.

The procedure described by Kobayasi[4] was followed. A and B sera which were used had titres 160 and 80 repsectively. As is illustrated in Table II, the bull carbohydrate equaled the one from hog (s. above) regarding this serological activity, whereas that of horse was incomparably inert.

TABLE II.

Agglutination of A and B erythrocytes by corresponding sera preliminarily treated with the carbohydrates.

Group of serum	Substance \ Dilution of substance	Orig. sol (1%)	×10	×10²	×10³	×10⁴	×10⁵
A	Bull carbohydrate (Prep. I)	±	++	+++	+++	+++	+++
	Horse carbohydrate	++	+++	+++	+++		
B	Bull carbohydrate (Prep. I)	−	−	−	−	−	++
	Horse carbohydrate	−	−	±	+++		

This work was aided by a grant from the Department of Education (through the Grant Committee of the National Research Council), which is gratefully acknowledged.

References.

1) Sinokawa, Tohoku J. Exp. Med., this volume, p. 167.
2) Utusi, Huzi, Matumoto and Nagaoka, ibid., this volume, p. 175.
3) Kobayasi, Tadasi, J. Bioch., 1941, **34**, 145.
4) Kobayasi, Tadasi, ibid, 1941, **33**, 369.
5) Satoh, Hamasato, Tetu Kobayasi and Sinokawa, Tohoku J. Exp. Med., 1943, **46**, 189.
6) Lüdtke, Biochem. Z., 1929, **212**, 422.

The Tohoku Journal of Experimental Medicine, Vol. 50, Nos. 3 & 4, 1949.

Hexosamine Compounds in Human Gastric Juice.

First Communication: Fractionation of Gastric Juice*.

By

Hirosi Tiba
(千葉 博)
(From the Medico-Chemical Institute, Tohoku Imperial University, Sendai. Director: Prof. H. Masamune.)

and

Nobumiti Teramura and Aizi Gotoh.
(寺邑 信倫) (後藤 愛爾)
(From the Medical Clinic of Prof. T. Kurokawa, Tohoku Imperial University, Sendai.)

(Received for publication, March 30, 1946)

One decade and a half ago, Babkin and his collaborators[1] pronounced that "dissolved" mucin involving Levene's mucoitinsulfuric acid is present in canine gastric juice[2] and furthermore that free mucoitinsulfuric acid unassociated by protein is not present in this secretion. On the other hand, it has been known among serologists since 1927 that the blood group substances are secreted in human gastric juice[3] and recently Witebsky and Klendshoj[4] isolated a "carbohydrate-like" substance possessing B property from gastric juice of patients belonging to this group. These group substances might be also conjectured to contain hexosamine, when analogized from what has been revealed with respect to those from other sources[5].

Taking the above informations into consideration, we have made trials to separate the individual hexosamine compounds in a pure state. The procedure which will be described in the experimental part appeared most simple and expedient. From each A and B gastric juice were obtained three kinds of substance. Two of them resembled mutually in biuret-negativeness, in strong Molisch-positiveness and in giving a red dye with dimethylaminobenzaldehyde, when heated in advance with alkali (Osaki and Turumi procedure[6]), but diverged in inhibiting isoag-

* The 98th report of Biochemical Studies on Carbohydrates, Masamune.

glutination of erythrocytes extremely, one exhibiting only very faint activity probably due to contamination by the other. The third substance gave all those chemical reactions but possessed no anti-agglutinative property. Detailed studies, chemical and physical, of the preparations will be accounted for in following papers. It should be worthy to note here that we could not demonstrate the naphthoresorcinol reaction for hexuronic acid (Neuberg and Saneyoshi) neither in those final substances nor in the crude fractions nor in the starting material which had been precipitated from condensed gastric juice with alcohol.

EXPERIMENTAL.

Procedure of Fractionating Hexosamine Compounds in Gastric Juice.

Human gastric juice was collected from patients by the aid of a caffeine test solution (Katsch method). The diluted juice (pH 1.2–4.6) from each patient was first percolated (repeatedly two or three times) through a gauze and then made clear by filtration through a creased paper, after pH had been shifted, when necessary, to 4.2–4.6 with a saturated sodium acetate solution (turbidity increase was hardly noticed). The filtrate was evaporated to syrupy consistency under reduced pressure and 95% alcohol was added. The precipitates were washed with absolute alcohol and with ether and placed in a vacuum desiccator. The dried powder[7] amounted to 144 and 112 mgm. in averages respectively per 100 cc. of A and B juice. The group was determined on these materials. Later operations will be described taking a typical example started with those obtained from A group juice and united.

1.9 gm. of the powder were mixed well with 20 cc. of ethylene glycol and centrifuged over and over to get a transparent supernatant liquid. The centrifugate was again treated with 10 cc. of glycol similarly and the residue (α-Fraction) was washed with alcohol and ether and set aside —900 mgm. To the fluids combined was added as much absolute alcohol as to throw down all the precipitable substances. The latter were separated and after washing and drying as above (320 mgm), again agitated with three times the weight of water. The mixture was acidified to pH 4.2 with drop by drop of 30% acetic acid and prolonged centrifugation exerted. The supernatant clear liquid was condensed to half the volume at a low temperature and 10 volumes of glacial acetic acid (96%) added. The appearing precipitates were centrifuged, washed once with glacial acetic acid, many times with absolute alcohol to eliminate the whole acetic acid and finally with ether and dried (β-Fraction)—180 mgm. From the supernatant fluid here was thrown down a further fraction (γ-Fraction) by means of

98% alcohol (7 volumes were required for complete precipitation), washed with alcohol and ether as before and dried —100 mgm.

The yields of the β and γ fractions diverged widely from one run to another but the amounts per 100 cc. of gastric juice of the final substances obtained in the manner below were resembling independently from the run and also from the blood group.

Purification.

β-Fraction (180 mgm.). 10 cc. of water were used for dissolution. Although centrifugation was repeated, the supernatant liquid remained turbid. Hence this was precipitated with alcohol (95%, 60 cc.), and after washing with alcohol and ether and drying, again taken up in water (5 cc.). This time a clear supernatant liquid was obtained on centrifugation. The substance separated and dried as above was then freed from glycol-insoluble impurities by the aid of 10 cc. of this solvent. Precipitates were gained from the clear solution again by alcohol (95%, 70 cc.) and washed and dried similar to above. Finally they were taken up (a perfect solution was given) in 5 cc. of water and shaken with 75 cc. of glacial acetic acid (96%) for two days on an apparatus. The undissolved part was collected, washed with glacial acetic acid once, with many changes of 95% alcohol and with ether and dried. White powder amounting to 70 mgm. was obtained (N.A.A.C.).

γ-Fraction (100 mgm.). It was taken up in 5 cc. of water and the insoluble residue centrifuged off. To the opalescent supernatant liquid, which was not to be readily clarified by centrifugation, were added 2 cc. of 30% acetic acid. The turbidity-causing substance was thereby changed into flocculent precipitates, which were separated off by centrifugation. Alcohol (60 cc.) was added to the clear supernatant liquid and what was precipitated was washed with alcohol and ether and dried. Thereupon followed thorough mixing with 10 cc. of glycol and subsequent centrifugation. The supernatant liquid here was also precipitated with 95% alcohol (70 cc.) and the precipitates washed and dried likely. A single repetition of glycol treatment sufficed to remove all the substance insoluble in this fluid. Non-coloured powder produced was 63 mgm. (A.A.C.).

α-Fraction (900 mgm.). This was not exhaustively purified:— 500 mgm. of this fraction were at first kneaded with a small volume of glycol and later agitated with the increased solvent (30 parts in total) and centrifuged. The centrifugate was washed with absolute alcohol, and dried after replacing alcohol with ether. 350 mgm. of brownish powder were yielded.

Chemical Properties and Serological Activity of the Products.

N.A.A.C.'s (Non-Antiagglutinative Carbohydrate).

They did not dissolve in water (only swelled), but did when neutralized. Biuret, ninhydrin, aniline acetate (for pentose), naphthoresorcinol (for hexuronic acid) and ammonium molybdate (for phosphorus) tests resulted all in negative. They showed intensive Molisch reaction (violet coloration). Dimethylaminobenzaldehyde reaction (Osaki and Turumi procedure) occurred plainly after heating of their alkaline solution but scarcely when this preliminary treatment was omitted. Hydrolysable sulfur ($BaSO_4$ method) was detected. Arginin (Sakaguchi) was present but histidine (Ehrlich diazo) absent.

The anti-isoagglutinative power was measured after Kobayasi[8]. The employed A and B sera possessed titres 160 and 80 respectively. Both of the substances from A and B stomach juice showed group-specific activity only up to 1:1,000 dilution (10 times dilution of the original solution).

A.A.C.'s (Anti-Agglutinative Carbohydrate).

Qualitative chemical tests gave similar results as in the former polysaccharides. The substances from the both groups of stomach juices inhibited the agglutination specifically up to 1:1,000,000 dilution. The sera had the same titres as those in the preceding case.

The α-Fractions Purified with Glycol.

Biuret and Molisch reaction were shown. Direct Osaki and Turumi test, negative; the indirect (test for acetylhexosamine), positive. Hydrolysable sulfur was detected. Aniline acetate test for pentose was given although naphthoresorcinol test for hexuronic acid was not. The presence of phsophorus was proved. The substance was insoluble in water and even when shaken with a sodium carbonate solution, most of it remained undissolved and in this part was found a large quantity of phsophorus. The substance was also insoluble in dilute hydrochloric acid.

The serological assay indicated them non-anti-isoagglutinative.

A grant was given from the Department of Education (through the Grant Committee of the National Research Council) to aid this work, which is gratefully acknowledged.

References and Notes.

1) Babkin, Am. J. Surgery, New Series, 1929, **7**, 498. Webster, Tr. Roy. Soc. Canad., 1930, **24**, sec. 5, 199. Babkin, Canad. Med. Ass. J., 1931, **25** (New Series), 134. Webster and Komarov., J. Biol. Chem., 1935, **109**, 177.

2) Collected by sham feeding from dogs with gastric fistula and esophagotomy.

3) Brahn and Schiff, Klin. Wschr., 1929, 1523. Schiff, Über die gruppenspezifischen Substanzen des menschlichen Körpers, Gustav Fischer, Jena, 1931, 49.

4) Witebsky and Klendshoj, J. Exp. Med., 1940, **72**, 663.

5) Freudenberg and Eichel, Liebigs Ann., 1934, **510**, 240, and 1935, **518**, 97 (human urine). Landsteiner, J. Exp. Med., 1936, **63**, 185 (horse saliva). Landsteiner and Chase, ibid., 1936, **63**, 813 (commercial pepsin). Meyer, Smyth and Palmer, J. Biol. Chem., 1937, **119**, 73 (commercial mucin). Freudenberg et al., Sitzgber. Heidelberg. Akad. Wiss., Math.-naturw. Kl. Abh., 1938, **1**, 1 (human urine). Landsteiner and Harte, J. Exp. Med., 1940, **71**, 551 (commercial mucin and pepsin). Kobayasi, Ohhara, Sinokawa, Yasuoka and Adati, J. Bioch., 1941, **34**, 149 (human urine). Yasuoka, Tohoku J. Exp. Med., 1948, **49**, 213 (human urine). Sinokawa, ibid., this volume, 167 (mucus mucin of swine stomachs). Tiba, ibid., this volume, 281 (mucus of bull and horse stomachs).

6) Osaki and Turumi, Tohoku J. Exp. Med., 1947, **49**, 11.

7) This was coloured with methylene blue added to the caffeine test solution.

8) Kobayasi, J. Bioch., 1941, **33**, 369.

Experiments of Color Contrast on Excised Frog Eyes.

By

Kōiti Motokawa and **Kituya Iwama.**

(本川 弘一)　　(岩間 吉也)

(From the Physiological Laboratory of Prof. K. Motokawa, Tohoku University, Sendai.)

(Received for publication, April 20, 1949)

As reported in our preceding papers,[1][2] the excitability curve which represents the time course of electrical excitability of the retina after a brief illumination, shows generally three distinct humps R, G and B (see Fig. 1). In the present paper, we deal with the effect of colored light which precedes white light upon the excitability curve for the white light. The procedure of experimentation is shown schematically in the insets of Fig. 1, where the notation b-g. represents bluish green light, r-y. reddish yellow, Wh. white light and S electrical stimulation of one second in duration.

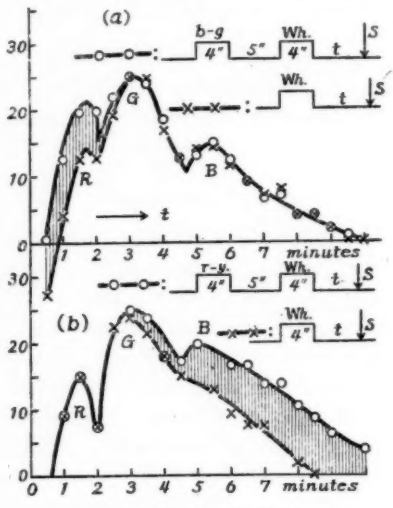


Fig. 1. Explanation in the text.

As can be seen in the figure, bluish green light preceding white one enhances selectively the R process, and reddish yellow light preceding white light increases mainly the B process. We know that red light alone gives rise to the R process.[2] Therefore, the selective enhancement of the R process stated above must be so interpreted that bluish green light succeeded by white light has caused a retinal change underlying a red sensation. The above findings, therefore, furnish us with physiological evidence of successive color contrast.

Literature.

(1) Motokawa, K. and Iwama, K., This Journal, 1949, **50**, 240.

(2) Motokawa, K. and Iwama, K., Ibid., 1949, **50**, 258.

The Tohoku Journal of Experimental Medicine, Vol. 50, Nos. 3 & 4, 1949.

Biochemical Studies on Carbohydrates.

XCIX. Hexosamine Compounds in Human Gastric Juice. Second Communication: A and B Group Specific Carbohydrates (A.A.C.).

By

Hirosi Tiba.

(千葉　博)

(From the Medico-Chemical Institute, Tohoku Imperial University, Sendai. Director: Prof. H. Masamune.)

(Received for publication, March 30, 1946)

Quantitative analysis revealed that purified A and B specific substances in human gastric juice (A.A.C. in the first communication[1]) are composed equally to each other, so far as the constituents arranged in Table I are concerned, and both of the monosaccharide constituents

TABLE I.

Analysis	In per cent		In equivalents per equivalent weight	
	A polysaccharide	B polysaccharide	A polysaccharide	B polysaccharide
Nrtrogen*	3.60	3.79	1.27	1.38
Glucosamine	36.3	35.1	1.0	1.0
Acetyl	8.66	8.04	0.99	0.95
Galactose†	34.7	35.3	0.95	1.00
Hydrolysable sulfur	0.54	0.64	0.08	0.10
Ash‡	2.4	0.76		

* Micro Kjeldhl method. † Indole, orcinol and diphenylamine I method gave concordant results. ‡Mirco analysis.

involved in the equimolecular ratio. However, they diverged in optical rotatory power in water as follows.

A substance:

$$[\alpha]_D^8 = \frac{-0.68 \times 1.431}{0.0233 \times 1.003 \times 1} = -41.2°$$

B substance:

$$[\alpha]_D^9 = \frac{-0.79 \times 1.389}{0.0178 \times 1.002 \times 1} = -61.3°$$

The hexosamine and hexose in both of these polysaccharides were identified with glucosamine and galactose in a similar manner as in the 97th investigation of this series[2].

120 mgm. A polysaccharide:
35 mgm. glucosaminepentabenzoate (the crude, 43 mgm.).
F. P., 215°C. Mixed test, no depression.
N (micro Kjeldahl), 2.06% (calc., 2.00%).

$$[\alpha]_D^{10}\text{ (in pyridine)} = \frac{0.515 \times 1.1138}{0.0128 \times 0.983 \times 1} = +45.1°.$$

26 mgm. galactosemethylphenylhydrazone (the crude, 31 mgm.).
F.P., 188.5–189°C. Mixed melting point, 188.5–189°C.
N (micro Dumas), 9.69% (calc., 9.85%).

150 mgm. B polysaccharide:
35 mgm. glucosaminepentabenzoate (the crude, 45 mgm.).
F. P., 215°C. Mixed test, no depression.
N (micro Kjeldahl), 1.97% (calc., 2.00%).

$$[\alpha]_D^{12}\text{ (in pyridine)} = \frac{0.58 \times 1.164}{0.0151 \times 0.985 \times 1} = +45.4°.$$

24 mgm. galactosemethylphenylhydrazone (the crude, 32 mgm.).
F.P. 188°C. Mixed melting point, 189°C.
N (mirco Dumas) 9.91% (calc., 9.85%).

The present substances are the second couple of polysaccharides displaying different specific activities in spite of their being similar in composition (qualitative as well as quantitative) regarding at least the sugar components. The first couple was those from urine which were analyzed by Yasuoka[3]. In both pairs, B substances rotated polarized light more to the left and on the other hand the molecule is not fulfilled by the analyzed components altogether. An unknown constituent or constituents occupy even nearly 30% of the molecule in the present polysaccharides. It is considered that the unknown play an important role in inhibiting the specific agglutination as most probably does the mode of glucosidic union (cf. Yasuoka with respect to the enzymic cleavability).

In the next, the present A specific carbohydrate resembles those from stomach mucus of swine and cow[2] as well as that from swine gastric mucosa[4] in connection with the quantitative ratio between glucosamine and galactose, but diverges from the mucus polysaccharides in optical power[5] and solubility in glacial acetic acid and from the mucosa polysaccharide in solubility not only in glacial acetic acid but also in water. These dissimilarities may be ascribed partially, to say the least of it, to the type of linking the monosaccharides and the number of the unit disaccharide in a molecule. But whatever it may be, it is now a fact indisputable that isoagglutination-inhibitory carbohydrates, which belong to the same blood group and are of like potency, can take different structures and polymerization or association states.

The Government Grant Committee of the National Research Council gave a grant to aid this investigation, which is gratefully acknowledged in this place.

Bibliography and Notes.

1) Tiba, Teramura and Gotoh, Tohoku J. Exp. Med., this volume, p. 287.

2) Tiba, ibid. this volume, p. 281.

3) Yasuoka, ibid., 1948, **49**, 213. Hexosamine in A polysaccharide of urine was identified with glucosamine by Freudenberg and Eichel (Liebigs Ann., 1935, **518**, 97). The exact nature of the one in B polysaccharide from the same source has not been as yet established but it appears to be also glucosamine when analogized from the finding in the present work.

4) Sibuya, Tohoku J. Exp. Med., in press.

5) The A specific mucus polysaccharides are less rotatory despite the larger percentage of the proper carbohydrate moiety in their molecule.

Studies on Melanin.

I. The Relationship between Melanin Production and Dioxybenzenes.

Preliminary Report.

By

Atsushi Nakajo.

(中條　敦)

(From the Department of Dermatology, Faculty of Medicine, Tohoku University, Sendai. Director: Prof. M. Ito.)

(Received for publication, April 21, 1949)

In order to make clear how the pigment is produced in human skin' the auther made following experiments.

Three isomers of dioxybenzenes, hydroquinone, resorcinol and pyrocatechol were injected intradermally to black goldfish (Kurodemekin), using saline solution as controls. Further experiments were made with above mentioned dioxybenzenes in combination with various hormones and vitamins. Results:

1. By intradermal injection of hydroquinone solution (0,5%), bleaching of melanin in the epidermis and in the scales was found. Dopa reaction turned negative after the decoloration had been completed, but melanin in the chromatophore remained unchanged. Two isomers, resorcinol and pyrocatechol, however, were proved ineffective in spite of the stronger reducing potency of the latter than hydroquinone.

2. Vitamin C, B-complex and cortical hormon of adrenals exerted no inhibition on melanin production.

3. Optimal temperature for this process is 15–20° C., and ultraviolet ray accelerates both depigmentation and repigmentation.

4. The mechanism of the depigmentation by hydroquinone cannot be explained by mere reduction of melanin, but rather by the special suppression against dopa oxydase through its anti-alkalisations potency.

This article was abstracted from the original article in Hifuka-Seibyoka Zasshi (Jap. J Derma. & Ven.), 1942, **51**. The details will be published in a later issue of this journal.

The Tohoku Journal of Experimental Medicine, Vol. 50, Nos. 3 & 4, 1949.

Comparative Studies on the Brain-Waves of Various Animals in their Natural Resting States.

By

Kōiti Motokawa.
(本川 弘一)

(From the Physiological Laboratory of Prof. K. Motokawa, Tohoku University, Sendai.)

(Received for publication, September 6, 1946)

Brain-waves can appear quite differently under different conditions even in one and the same animal, and the same potential changes can appear in quite different patterns when recorded by apparatus of different characteristic. It is necessary to make experiments under comparable conditions of animals and with the same apparatus for the purpose of comparative studies. In view of the fact that these conditions are fulfilled in few of the works ever published, I have undertaken the present investigation.

Method.

Most experiments have hitherto been carried out under some unnatural conditions such as narcosis, surgical treatments, restraint etc., and it goes without saying that electroencephalograms (eeg) taken under such conditions cannot be compared with those of normal man in his natural resting state.

In order to take eeg under conditions as natural as possible, the potential changes were led off from the scalp of animals which had been so trained as to adapt themselves to the procedure. My assistant kept the animal gently on his knees and trained it till it could remain motionless at least during the recording of eeg. The lead-off-electrodes of silver were placed upon clean-shaved spots of the forehead and the occiput, and the electric contact was made with electrode-paste rich in NaCl. This wide bipolar lead is, of course, little suitable for finding any local difference in potential pattern, but it is unavoidable when working on an unopened skull.

The recording apparatus was an electro-magnetic oscillograph of

Yokogawa combined with four stages resistance-capacity-coupled amplifier, whose frequency-characteristic is shown in Fig. 1. This characteristic is very skew in such a manner that amplification decreases steeply, then more slowly as the frequency increases. An amplifier with such a skew characteristic as this may seem to be little adequate for our purpose to record potential changes faithfully, but strange to say, it can be more adequate for recording them from the scalp than an amplifier with an ideally homogeneous characteristic throughout the whole range of frequencies.

The reason for it has been given in the work of Motokawa and Iwama[1]).

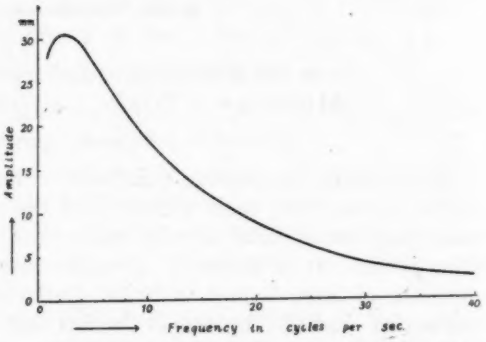


Fig. 1. Frequency-characteristic of the amplifier used in this experiment.

According to these authors, the polarization-capacity of the skin acts on the eeg led off from the scalp in such a manner that the lower the frequency of waves the stronger the reduction of the amplitude caused by the capacitive effect. However, this effect is nearly compensated by the skew characteristic of the amplifier so that waves of various frequencies are reproduced in almost natural proportion of amplitudes in eeg.

Animals used in this experiment were dogs, cats, rabbits and guinea pigs. Rats were not used because of their too small heads to lead off potentials in the same way as in other animals. Some experiments were made on a monkey which, however, could not be trained and had to be narcotized with dial. The result is, of course, not comparable with other results on non-narcotized animals, so that it has been omitted from the present report.

Results.

It was found that potential changes of man and animals were of the same order of magnitude under the comparable conditions of this experiment. Greater values amounting to several hundred μV found by other investigators must be due to the direct leading off from the brain. From the scalp such a large value has never been obtained.

Individual differences were found not only in the magnitude of potential changes, but also in potential patterns. They were sometimes so great that it was difficult to guess to which species the eeg in question

belonged, especially between species akin to each other like cats and dogs. Eeg of man could usually be distinguished easily from those of animals, in general, but sometimes with difficulty from those of some dogs. The potential patterns of eeg for any species covered a wide range of variety owing to individual differences and consisted of elements more or less common to all species. Therefore, it will be only in a statistical sense that we can distinguish some characteristics of species with respect to eeg.

As the norm for the given species I selected the type of patterns, which had most frequently appeared, and compared it with those types selected

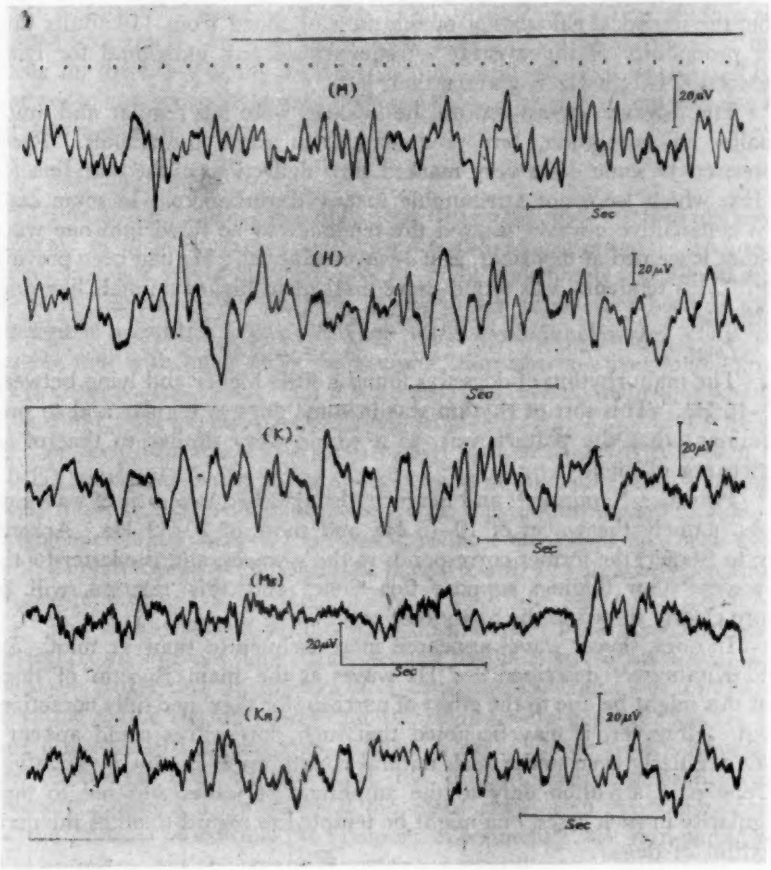


Fig. 2. Electroencephalograms of man and animals in their natural resting states. (M): Man, (H): Dog, (K): Cat, (Ms): Guinea pig, (Kn): Rabbit.

on other species. The typical records for man and animals are illustrated in Fig. 2.

Man.

Since Berger's discovery, the eeg of man has been so extensively studied that there remain few things to add here. The main rhythm of man's eeg is, as well-known, the rhythm of about 10 Hz or cycles per sec (α-rhythm or waves). The frequency of this sort of waves is almost constant; my strict statistical study shows that its time-variation remains always within 10 per cent at any individual. The individual variation of the α-frequency is also small; the author has been able to conclude from the statistical calculation of the data obtained from 118 adults that the probability of the average α-frequency of any individual for lying between 8.4–11.9 Hz is greater than 0.95.

The β-waves superposed on the α-waves were less regular and much smaller in amplitude, but predominant in some individuals. There appeared in some cases very marked slow deflections of the base-line (1-3 Hz) which were not attributable to any disturbances. In some cases two consecutive α-waves showed the tendency to be fused into one wave so that it seemed at first sight, as if δ-waves of about 5 Hz had been prevailing. This tendency was found more marked in higher animals like dogs and cats.

Dogs.

The main rhythm of dogs was found a little higher and lying between 10–16 Hz. This sort of rhythm was in some dogs so regular and so predominant that the pattern was, as a whole, very similar to that of an α-rich eeg of man.

Prawdicz-Neminsky[2] and Berger[3] distinguished two sorts of waves on dogs, namely the waves of 10–15 Hz and those of 20–32 Hz. According to Berger, the former corresponds to the α-waves, and the latter to the β-waves. Our findings support this view, and this relation will be more clearly shown in the analysis of eeg.

In dogs, slower waves appeared more frequently than in man. Ito and Kitamura[4] described 9–7 Hz waves as the main rhythm of dogs, but this might be due to the effect of narcosis, for they used only narcotized dogs. However, it may be noted that such slow waves could appear a little regularly even under such natural conditions as in our investigation. If one paid attention only to the amplitude of waves and not to their regularity in such cases, one might be tempted to regard them as the main rhythm of dogs.

Cats.

The eeg of cats are generally very similar to those of dogs. The

average frequency of the main rhythm is about the same in both species, but the range of variation seemed to be a little wider in cats than in dogs.

The waves of higher frequencies superposed upon the main rhythm must correspond to the β-waves of man, because they increased in frequency as well as amplitude upon excitation just ilke in man. Derbyshire, Remple, Forbes and Lambert[5] reported that waves very similar to those of man were observed in non-narcotized cats, and Rheinberger and Jasper[6] pointed out that the pattern of eeg of non-narcotized cats depended greatly upon the excitation level of the brain, and showed that the β-waves of 20–50 Hz would last for a considerably long time, when once initiated by some excitation. The tendency to slower rhythm was found stronger in cats than in dogs, especially when the cats were calmly lying on the knees of my assistant. This fact is to be so interpreted that the eeg of cats are more akin to those of rabbits, whose main rhythm consists of slow waves of 3–7 Hz.

Rabbits.

The 3–7 Hz waves have been regarded as the main rhythm of rabbits because of their consistent appearance and great amplitude. The trace of these slower waves have been found in higher animals and also in man, as stated above. So it seems as if the slower waves had become more dominant in rabbits because of their lower evolutional stage. This idea agrees well with other facts, for instance, that slower waves predominate in the eeg of a new born. But it is necessary to investigate further whether this could be a general law with regard to eeg.

As seen from the record (Kn) in Fig. 2, there appeared waves of 15-30 Hz superposed upon the slow main rhythm, and they were sometimes even more regular and more steady than the slower waves, so that we might have taken them as the main rhythm, if the record had been taken with a greater speed of photographic paper or with an amplifier less sensitive to slower waves.

The brain-waves of rabbits have been studied by a great number of investigators, but the agreement of their results is not satisfactory. This must be partly due to differences of experimental conditions, but partly also due to the circumstances that the rhythms are generalll less regular and less consistent in these animals than in other higher ones. Kornmüller[7] reported 2–4 Hz waves at the occiput, whereas Bartley and Bishop[8] regarded 5 Hz waves as the main rhythm in the same region of the cortex. According to Ectors' experiment[9] on non-narcotized and untied rabbits the potential patterns were subject to constant variation even at one and the same region of the cortex without any sign of changes in outward appearance.

In the experiment of Marinesco, Sager and Kreindler[10] on non-narcotized rabbits the predominant waves in the region from the area striata up to the sulcus centralis were 2–6 Hz waves, on which 15 Hz waves were superimposed at the gyrus centralis, and the 11 Hz waves were the main rhythm at the gyrus centralis anterior.

These authors considered that the rhythm common to the whole cortex, like the α-wave of man, was 2–6 Hz waves at the rabbit, and this agrees well with the result of my experiment.

Guinea pigs.

The general pattern of eeg of guinea pigs was very similar to that of rabbits, but the frequency of the main rhythm was a little higher and lay between 6–8 Hz. The record (Ms) in Fig. 2 shows how regular and conspicuous the waves of higher frequencies superimposed upon the slower waves are. They could be even more regular and persistent than the slower waves.

Jasper, Bridgman and Carmichael[11] described 8 Hz waves as the main rhythm, and Ito and Kitamura 7–9 Hz. As confirmed also in my experiment, these slower waves would disappear and return without any apparent cause.

Discussion.

Berger distinguished three kinds of brain-waves called α, β and δ. But American authors proposed to describe the waves with their approximate frequencies without making use of any particular notation, becasue Berger's classification does not apply to the eeg of animals. The question has often been raised, what sort of waves of animals corresponds to the α-, and β-and δ–waves of man. And we have already raised the question whether the main rhythms of man and the animals investigated correspond to one another. To answer this question, we must know the properties of the main rhythms common to all species as well as those not common to them.

The common properties are 1. that they can be detected more or less at every region of the cortex, 2. that they are usually more regular and greater in amplitudes than any other kinds of waves, and 3. that they are reduced in amplitude on excitation. The properties not common are above all the range of frequencies covered by the main rhythms and the degree of dominance over other kinds of rhythms. The main rhythm of man, dogs and cats are so near in their frequency-ranges and dominance that we might be justified to assume a correspondence among them, but there is some doubt about the correspondence between the α-rhythm of man and the main rhythms of rabbits and guinea pigs for the following

reasons: 1. Such slower waves as correspond to the main rhythms of rabbits and guinea pigs can be found also in man and other animals, and appear more frequently in the order: cats, dogs and man, 2. in some cats, slower waves predominated in their resting states, 3. the main frequency of the rapid waves superposed upon the main waves of rabbits and guinea pigs falls within the limits of the frequencies of the main rhythms of cats and dogs and 4. the analysis of eeg, which will be described below, suggests the correspondence of the α-rhythm to the rapid waves of rabbits and guinea pigs, rather than to their slow waves.

It seems appropriate to assume that the solw waves correspond to the δ-waves of man which appear at earlier stages of ontogenesis, in normal sleep and under pathological conditions of the brain. It is interesting that the slow waves indicative of imperfect functional state of nerve cells predominate in phylogenetically lower animals such as rabbits and guinea pigs. One may be tempted to make a hypothesis that the higher the evolutional stage of creatures the higher the frequency of the main rhythm of their brain-waves, but such a hypothesis must stumble at once over the following difficulties: 1. The frequency of the main rhythm is lower in man than in dogs and cats, and 2. in rabbits and guinea pigs, 10–20 Hz waves can appear regularly and with large amplitudes.

It is, therefore, necessary to study the whole pattern of eeg instead of any particular kinds of waves, if one will elucidate the evolutional significance of eeg. For this purpose such a method of harmonic analysis of eeg, as has been adopted by Grass and Gibbs[12], would be of great advantage; but in view of the circumstances that such an analyser is seldom available in most laboratories, a smipler method of analysis accessible to every investigator is desirable. I propose the following method which needs no particular apparatus, but only simple measurements of wave-lengths with a measure. The procedure of analysis is as follows: A given lenght, say, one meter of record, is taken to measure the wave-lenghts, beginning with the slowest rhythm in the record, for instance, 2–3 Hz waves. The values are expressed in m sec by comparing the wave- length with the time-marks in the record. The same measurement is then carried out on the waves of the second order, i.e. the waves which are superposed on the slowest waves measured already, then on the waves of the third order and so on. The so obtained values of wave-lengths for various kinds of waves are now classified into a number of groups equally spaced along the logarithmic scale of wave-lengths, and with so classified values a histogram is constructed in the usual manner.

The histograms obtained by such analysis of the records in Fig. 2 are illustrated in Fig. 3. It must be noted that in this analysis no account has been taken of the amplitude of waves merely for the sake of simplicity

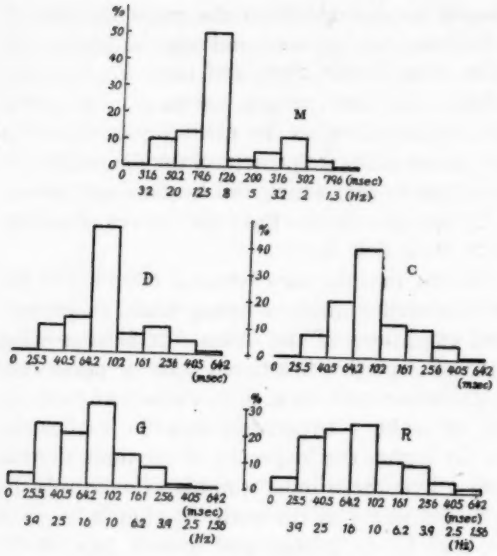


Fig. 3. Analysis of eeg of man and animals. The ordinate of histograms indicates how frequently the waves of the frequency-range given by the abscissa appear in a given length of records, and the abscissa gives the logarithm of the wave-lenth in m sec or the corresponding value of frequency.

M: Man, D: Dog, C: Cat, G: Guinea pig, R: Rabbit.

of analysing procedure. The curves or histograms indicate, therefore, how frequently the waves of given frequencies appear, but not how great their amplitudes are, as in the harmonic anlysis. If every kind of waves were distributed homogeneously in eeg, the curve would be expressed by such a steadily decreasing function with increasing wave-length as $y=c/\log x$, where y represents the ordinate of the curve, x the wave-length and c a constant, because the number of waves existing in a given length of record should be then proportional to their frequency or inversely proportional to their wave-length. This relation must be taken into account in the interpretation of our histograms. The curves of dogs and cats increase at first and then decrease, as the wave-length increases, and the maximum so established corresponds to the frequency-range of the main rhythm. The maximum of the curve for the guinea pig corresponds to the same frequency-range as in the dog and the cat, and in the curve for the rabbit, there can be found a trace of a maximum corresponding to the same frequency-range. If the waves appearing most frequently are regarded as the main rhythm, rabbit and guinea pig have the same main rhythm as cat and dog. If the waves with the greatest amplitude are chosen as the main rhythm as has been done above, the frequency-range of the main rhythms does not coincide with each other.

The sharpness of maxima is greatest in man, and least in rabbits, other animals ranking in the order: dog, cat and guinea pig between man and rabbit with respect to this property. It seems as if this property

of the curve could serve as a measure for the evolution of the central nervous systems.

In the histogram of man two distinct maxima can be observed, and this is also the case with the histogram of the dog, though less distinct. The gap between the two maxima is caused by scant appearance of waves ranging between 10–5 Hz in man and dogs, and this means that any gradual transition from α to δ hardly occurs in normal man. If Berger's classification is appropriate, it might be expected that three maxima could be distinguished in the histogram corresponding to the α-, β-and δ- groups, and in fact, it is possible to take at least the histogram of man as separated into three groups, i.e. a higher, an intermediate and a lower frequency-group if the rectangle of the histogram covering the α-range is regarded as an independent group. This holds good to some extent also for dogs and cats, but not guinea pigs and rabbits. The transition from the α group to the β group is more gradual in animals than in man, and such a classification as Berger's is no more possible in such lower animals as guinea pigs and rabbits, whose histogram appears to be a mere mass without any differentiation. The evolutional stage of animals is expressed in this way evidently by the degrees of differentiation of their histograms.

Summary.

In view of the circumstances that eeg of various animals have been studied with too different methods and under too different conditions to enable their comparative studies, the author tried to investigate them with one and the same method and under comparable natural conditions of man and animals, and obtained the following results:

1. The average amplitude of eeg taken from the scalp did not greatly differ in magnitude at man, dogs, cats, rabbits and guinea pigs.

2. The range of frequencies covered by the predominant waves was 8.4–11.9 cycles per sec. or Hz in man, 10–16 Hz in dogs and cats, 6–7 Hz in guinea pigs and 3–6 Hz in rabbits.

3. The correspondence among these main rhythms was studied from the phylogenetical point of view, and it could be concluded that the α-rhythm of man corresponded to the main rhythm of cats and dogs of 10–16 Hz, but not to the slower main rhythm of guinea pigs and rabbits.

4. A simple method of analysis of eeg was proposed. The result of analysis indicated that the higher the animals the more distinctly the frequencies of waves were differentiated.

References.

(1) Motokawa, K. & K. Iwama, Tohoku J. exp. Med., 1947, **49**, 89.
(2) Prawdicz-Neminsky, W.W., Pflügers Arch., 1925, **209**, 363.

(3) Berger, H., Arch. f. Psychiat. u. Nervenkr., 1929, **87**, 527.
(4) Ito, G. & K. Kitamura, Tohoku J. exp. Med., 1939, **37**, 106.
(5) Derbyshire, A.J., B. Rempel, A. Forbes & E. F. Lambert, Am. J. Physiol., 1936, **116**, 577.
(6) Rheinberger, M.B. & H.H. Jasper, Am. J. Physiol., 1937, **119**, 186.
(7) Kornmüller, A. E., Die bioelektrischen Erscheinungen der Hirnrindenfelder, 1937, Leipzig, Georg Thieme.
(8) Bartley, S.H. & G.H. Bishop, Am. J. Physiol., 1933, **103**, 173.
(9) Ectors, L., Arch. internat. Physiol., 1936, **43**, 267.
(10) Marinesco G., O. Sager & A. Kreindler, Bull. Acad. Med., Paris, 1938, **119**, 360.
(11) Jasper, H.H., C.S. Bridgeman & L. Carmichael, J. of exp. Psychol., 1937, **21**, 63.
(12) Grass, M. & A. Gibbs, J. Neurophysiol., 1938, **1**, 521.

The Tohoku Journal of Experimental Medicine, Vol. 50, Nos. 3 & 4, 1949.

On the Mechanism of Periodic Excitability of Nervous Tissue.

By

Kōiti Motokawa.
(本川 弘一)

(From the Physiological Laboratory of Prof. K. Motokawa, Tohoku University, Sendai.)

(Received for publication, September, 6, 1946)

There are at least two types of excitation, periodic and non-periodic. The most appropriate example for the former is the excitation of cortical cells, whose periodicity can be observed directly in brain-waves, and that for the latter is the response of the peripheral nerves to a brief induction shock. It is to be noted that the term "periodic" is used here not in such a strict sense as for physical systems, but as a synonym of a less rigorous term "rhythmical"; for the periodicity of a living tissue cannot be so regular as that of a physical system.

Any periodically excitable tissue may show automatic or spontaneous activity like the cortical cells, or may be non-automatic like the nerve cells in the spinal cord or the ganglions. Peripheral nerves which are non-periodically excitable under normal conditions can show rhythmical excitation under certain conditions. Katz[1] showed that the nerves of frogs acclimatized to a low temperature gave marked multiple response to a constant current, and Fessard[2] observed the same phenomenon at room temperature on frog's excised nerve which had been soaked in cold Ringer for many hours.

It is of great importance for the mechanism of periodic excitability that nerves which normally give no or very little repetitive response get periodic excitability when they are deprived of ionized Ca either by injection of Na Citrate or by soaking the isolated nerves in Ca-free Ringer (Katz, Fessard, Suzuki[3], Monnier & Coppée[4], Coppée[5] and others). The nerves so treated not only show repeated response to a constant current but also can fire off spontaneous discharges.

The distinction between automatically periodic systems and non-periodic systems is so obvious that there is no room for discussion. It is, however, a matter for consideration, whether such a system as gives

repeated response only during the initial stage of constant current flow or during a short period after the break of the current, should be regarded as a true periodic system. If periodic excitability should be taken as a property belonging to the excitable tissue but not to its milieu, it is difficult to understand that such a system should show its periodic property only under special external conditions, such as long duration of the stimulating current. If this is to be interpreted in such a manner that the periodic property is produced by the current and disappears after its withdrawal, such a temporary property should be distinguished from true periodic excitability inherent to the tissue. If the repetitive response has its cause in the repeated excitation by the long lasting effect of the current, as suggested by Katz, this must be due to the rapid recovery from refractoriness and the comparatively slow accommodation of the tissue. The refractory period and accommodation are properties inherent more or less to all kinds of tissue and imply no trace of periodicity in themselves.

At any rate, we have ample ground to distinguish such a system from true periodic systems. I propose to designate such a system a " quasi-periodic " system for the following reasons: 1. It possesses physiological properties intermediate between non-periodic and periodic systems and 2. it begins to show rhythmic spontaneous activity by severer deprivation of Ca-ions. Hence, such a system should be regarded as an intermediate link between the two systems, though not yet truely periodic in nature.

A quasi-periodic system and a non-automatic periodic system are in some respects so similar that they can easily be confused with each other, but their behavior is in the other respects so different from each other that the laws established at one system do not always hold for the other. This is the reason why a number of controversies have arisen among investigators who could not distinguish both systems. In the following I describe experimental methods for distinguishing the one from the other:

Methods for Distinguishing Quasi-periodic Systems from True periodic Systems.

(1) *Method of E-Curves.*

Since the periodic excitability is inherent to a true periodic system and depends little on external stimulation, the periodicity must be made apparent with a stimulus of very low intensity. On the contrary, a non-periodic or quasi-periodic system will show no periodicity at all with such a weak stimulus because the repetitiveness of response is the result of strong and long lasting excitation.

A subthreshold stimulus generally causes an " excitatory state " or " etat d'excitation " which can be tested by a test-stimulus delivered at

verious intervals after the preceding or sensitizing stimulus. We obtain so-called curves of " etat d'excitation " or E-curves by plotting the reciprocal of the intensities of test-stimuli required just to attain threshold against intervals. The distinction between true periodic systems and the others appears very clearly in the E-curves; they take a periodic course at true periodic systems and a non-periodic course at non-periodic and quasi-periodic systems. It must, of course, be admitted that there can be a gradual transition from strongly damped oscillations to a non-periodic course.

The E-curves obtained by Monier and Coppée[4] on nerves in Ca-deficit were of exponential form or of the form of a strongly damped oscillation. Motokawa and Iwama[6] obtained typically periodic E-curves at the human retina. The sensitizing currents were passed through

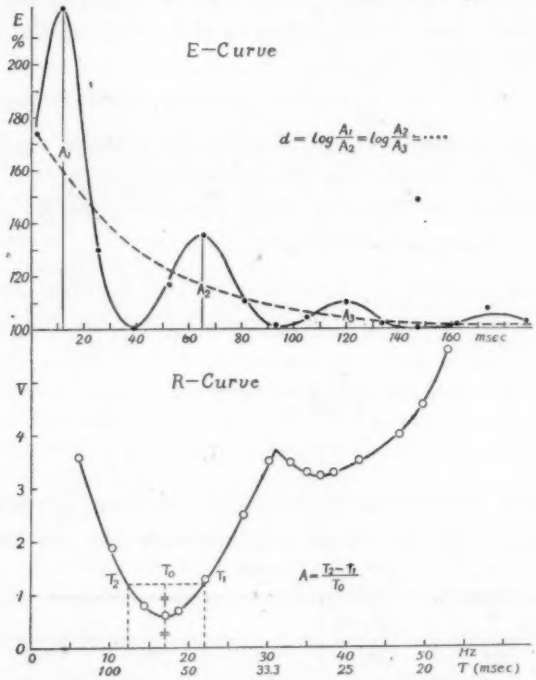


Fig. 1. E–curve (above) and R–curve (below). Ordinates for the E–curve: Reciprocals of threshold intensities of test-stimuli delivered at given intervals (abscissae) after a subthreshold sensitizing current, the value without the sensitizing stimulus being taken as 100.

Ordinates for the R–curve: Threshold voltage for alternating currents with frequencies in Hz or periods in m sec as given as abscissae.

the head of the subject by means of silver electrodes of 3×4 cm in size placed at the forehead (glabella) and the occiput, the electric contact being made with electrodepaste rich in NaCl. The index of excitation was the slightest possible light-sensation. The results were little altered by the form and duration of the sensitizing and the test-currents. An example is shown in Fig. 1. The excitability (reciprocal of threshold) is expressed in percentage, the excitability in the non-sensitized state being taken as 100. The amplitude of oscillation depended largely upon the state of adaptation of the eye; we obtained the most marked oscillation in a moderately light-adapted state.

The following experimental formula fitted most closely the data:

$$E=Ae^{-\frac{k}{t}}\{1+\cos(\omega t+\varphi)\}, \qquad (1)$$

where E represents excitability, A the maximum amplitude, t time and φ a phase-angle. The circular frequency ω is related to the period T of oscillation in the following way:

$$\omega=\frac{2\pi}{T}=\frac{2\pi}{55}=0.114 \text{ per m sec,}$$

where the period was put 55 m sec on an average. In this formula the damping coefficient is expressed by $1/k$ which is related to the well-known experimental measure of damping, i.e. the logarithmic decrement d in the following way:

$$d=\log_{10} e\frac{T}{k}=0.4343\frac{T}{k}. \qquad (2)$$

The values of logarithmic decrement of our E-curves are shown in Table I. If the value of k is calculated from the average value of d, 0.486 we obtain:

$$k=0.4343\times\frac{55}{0.486}=49.1 \text{ m sec.}$$

TABLE I.

Logarithmic decrement d of the E-curve. $d=\log_{10}\frac{A_1}{A_2}=\log_{10}\frac{A_2}{A_3}$, where the amplitudes of the first, second and third oscillation are denoted by A_1, A_2 and A_3.

No. of exp.	1	2	3	4	5	Average
A_1	129	158	114	100	102	
A_2	82	70	38	28	35	
A_3	26	25	10	12	10	
A_1/A_2	2.8	2.26	3.7	3.6	2.9	3.08
A_2/A_3	3.15	2.8	3.8	2.34	3.5	3.12
d	0.474	0.403	0.574	0.473	0.505	**0.486**

This value agrees very well with the value of k obtained from the data concerning strength-duration-relations as will be explained later.

On further scrutiny we found that small harmonic oscillations are superposed on the above mentioned fundamental oscillation of the E-curve. Hence the E-curve, when strictly taken, must be of the form:

$$E=e^{-\frac{t}{k}}\{A_0+A_1\cos(\omega t+\varphi_1)+A_2\cos(2\omega t+\varphi_2)+\dots\}\ .$$

It is to be regretted that the amplitude of oscillation in complete light- and dark adaptation is too small to allow an exact measurement of the logarithmic decrement, the frequency itself did not seem to be influenced greatly by the adaptation state of the eye.

(2) *Method of R-Curves.*

For a system vibrating under the action of a given alternating force the amplitude of oscillation is maximum, when the frequency of the driving force nearly equals the natural frequency of the system, and this phenomenon is known as "resonance." This relation applies also to the stimulation of excitable tissue with alternating currents. Coppée[5] studied this relation on nerves of cold blooded animals. Motokawa and Iwama[6] obtained at the human eye typical resonance-curves (R-curves) an example of which is illustrated in Fig. 1. Thresholds in volts are plotted as ordinates and the frequencies in Hz (to read from left to right) or the periods in m sec (to read from right to left) as abscissae. In this Fig. two minima are seen, one of which lies at the frequency of.17 Hz and the other at a frequency about 2 times as high as the former. In some cases three to four minima were observed and their frequencies were always in harmonic relations. This corresponds to the above mentioned fact that harmonic oscillations were found superimposed on the fundamental oscillation of the E-curve.

The period of the fundamental oscillation was, on an average, 56.24 m sec and agreed very well with the average value 54.85 m sec obtained from the E-curves. The flatness of the minima depends chiefly upon the damping in such a manner that the greater the damping the flatter the minima of the R-curve. Coppée expressed the flatness ("aplatissement") with the following measure: $A=\frac{T_2-T_1}{T_0}$, where T_1 and T_2 represent the periods corresponding to the threshold voltages two times as high as the minimum voltage, and T_0 the period corresponding to the minimum, as explained graphically in Fig. 1. The value of A was 2.32 for the eyes light-adapted to 950 lux, 2.49 for the completely dark-adapted ones, and 0.84 for those moderately light-adapted to 1 lux. These figures suggest that damping is least with the moderately light-

adapted eyes, or that the E-curve should oscillate most freely in this adaptation state. The observations on the E-curves mentioned above agree very well with this inference from the R-curves.

Now the question arises whether all tissues possessing a minimum in their threshold-frequency-curves may be regarded as belonging to true periodic systems. A number of investigators proved that curves for even normal nerves show a threshold minimum at a certain frequency of the stimulating current. According to Hill's theory[7], the process underlying such a minimum is not resonace but accommodation. This idea has been substantiated by the following experiment of Coppée: When the strength of the stimulation current was gradually increased instead of making it suddenly, a minimum resulted, whereas the minimum was very slight or entirely wanting in the case of a sudden make; this fact is to be interpreted as evidence that accommodation is the direct cause for the formation of a minimum, because this was seen only under such conditions as allow development of accommodation.

Coppée called the minimum formation due to accommodation "pararesonance" in order to distinguish it from true resonance. He maintains that both can be experimentally distinguished by examining whether or not a minimum disappears by a sudden make of alternating currents, and this has been confirmed by our experiments.

Periodic Excitability and Accommodation.

It is an important question, whether or not the slowness of accommodation in a tissue is a necessary condition for periodic excitability. Katz showed that the duration of repetitive response to a constant current increased in proportion to the accommodation constant λ, and it has been corroborated by Suzuki, Kurebayashi and Oku,[8] though it has not been decided to which system, quasi-periodic or true periodic, the nerves used in their experiments belonged. In the experiment of Motokawa and Iwama[9] the accommodation constant λ of the retina of man moderately light-adapted was smaller than that of his ulnar nerve which belonged without doubt to the non-periodic systems. More strange was that they obtained a smaller λ in the moderate light adaptation than in any other adaptation states, although periodicity was more marked in the former case than in the latter. Monier and Coppée found that there was no parallelism between periodic excitability and slowness of accommodation on nerves treated with Na Citrate, and concluded that the slowness of accommodation was no necessary condition for periodic excitability.

However Motokawa and Iwama pointed out that experimental values of λ could hardly be a faithful measure for accommoda-

tion, at least at periodic systems, because periodic excitability makes experimental values of λ apparently smaller. Therefore it is erroneous to conclude rapid accommodation from apparently small values of λ obtained at periodic systems. Katz's experiment suggests that accommodation is the more slowed down the more marked the repetitive responsiveness of the tissue. If there were no decreasing effect of periodic excitability upon λ, a very large value of λ would result at a periodic system like the human retina. A comparatively large value of λ over 100 m sec of the dark-adapted eye, the periodicity of which is less marked than the eye which is moderately light-adapted, may be interpreted as due to the weak decreasing effect upon λ.

The rheobase is generally lower for periodic systems than for non-periodic systems (Fessard and others). In our experiment the rheobase was lowest for the moderately light-adapted eye, the periodicity of which was clearest. This fact would be indirect evidence of slower accommodation in periodic systems; for a rapid and strong rise in threshold means rapid and strong accommodation, which can, therefore, never correspond to such a low rheobase as found at periodic systems. In the following section, I will theoretically explain large values of chronaxie found at periodic systems under the assumption that the speed of accommodation was infinitely small, and you will find a very satisfactory agreement between theory and experiment. This fact furnishes another indirect evidence of slow accommodation in periodic systems.

Periodic Excitability and Chronaxie.

It has been shown by a number of investigators that the value of chronaxie is generally large for a tissue inclined to repetitive response to a constant current. Iwama[10] found the chronaxie of the human retina greater in the moderate light adaptation than in any other adaptation states (s. Table VI). In order to elucidate the mechanism underlying this relation, a theory of electric stimulation for periodic systems has to be constructed, for all the theories hitherto presented apply to only non-periodic systems. However, it is obviously no simple matter to evaluate a general theory applicable to all possible cases; so I attempted to make a preliminary theory holding comparatively good for the data available at present.

When an electric current is passed through a living excitable tissue, it changes the "condition" of the tissue in such a way that, if the change be in the right direction and great enough, excitation results. Hill[7] called the "condition" the "local potential" V of the excitable tissue and assumed that it tends to decay exponentially to its initial value when the current is withdrawn. Hill's fundamental equation (3′) is:

$$\partial V = b\, I_\theta\, \partial\theta \quad e^{-\frac{t-\theta}{k}}$$

where I_θ is the current at the time θ, k the time-constant of excitation and b an arbitrary constant. This equation holds from time $t=\theta$ onwards.

Instead of Hill's exponential decay of the "local potential" let us assume such an oscillating course of decay as observed in the E-curve of eriodic systems and put

$$e^{-\frac{t-\theta}{k}}\,[1+\cos\{\omega(t-\theta)+\varphi\}] \text{ instead of } e^{-(t-\theta)/k}$$

in Hill's equation. Then the following equation is obtained for periodic systems:

$$\partial V = b\, I_\theta\, \partial\theta\; e^{-(t-\theta)/k}[1+\cos\{\omega(t-\theta)+\varphi\}] \qquad (3)$$

We have neglected accommodation for the reason discussed above and assumed that excitation results when the "local potential" V attains a definite level, say m. Integrating equation (3) from $\theta=0$ to $\theta=t$, we obtain the following equation with constant currents $I_\theta = I =$ constant, namely:

$$m=V-V_o = b\,k\,I(1-e^{-t/k}) + \frac{b\,k\,I_o}{1+k^2\omega^2}\,[\cos\varphi - k\omega\sin\varphi - e^{-t/k}\{\cos(\omega t+\varphi) - k\omega\sin(\omega t+\varphi)\}], \qquad (4)$$

where V_o is the resting value of the "local potential." If I_o is the rheobasic current we get, putting $t\to\infty$:

$$m=V-V_o = b\,k\,I_o + \frac{b\,k\,I_o}{1+k^2\omega^2}\,(\cos\varphi - k\,\omega\sin\varphi). \qquad (5)$$

Putting equation (4) equal to (5) we obtain threshold current I in terms of its duration:

$$I=\frac{I_o\,(1+k^2\omega^2+\cos\varphi - k\,\omega\sin\varphi)}{(1+k^2\omega^2)(1-e^{-t/k})+\cos\varphi - k\,\omega\sin\varphi - \{\cos(\omega t+\varphi) - k\,\omega\sin(\omega t+\varphi)\}\,e^{-t/k}}. \qquad (6)$$

The circular frequency ω in this equation is known from E- and R-curves and k is obtainable from E-curves. Hence, the validity of this equation can be tested by experimental data on the well-known strength-duration-relation, if the phase angle φ is appropriately chosen. The time-constant k was, in fact, regarded as an arbitrary constant and chosen in such a way that the equation fitted the experimental data as closely as possible. The so determined value of k agreed actually very well with the value obtained from E-curves. The observed and calculated values of I for various values of t are given in Tables II, III and IV (compare obs. values with those calculated after Motokawa's theory).

The agreement may be regarded as satisfactory, but taking into account that the strength-duration-relation can be fitted by any theory

TABLE II.

Strength-duration-relation at the human retina in the moderate adaptation (1 lux).
r: Difference between obs. and cal. values.

Duration in m sec	Threshold in volts									
	Obs.	Motokawa's theory			Hill's theory			Weiss's formula		
		Cal.	r	r^2	Cal.	r	r^2	Cal.	r	r^2
3.24	10.5	10.9	−0.4	0.106	10.6	−0.1	0.01	10.37	0.13	0.017
4.62	7.83	7.7	0.13	0.017	7.62	0.21	0.044	7.52	0.31	0.096
7.37	4.86	4.9	−0.04	0.0016	4.94	−0.08	0.0064	5.02	−0.16	0.0256
10.1	3.91	3.69	0.22	0.048	3.74	0.17	0.0289	3.88	0.03	0.0009
12.85	3.46	3.02	0.46	0.211	3.04	0.42	0.176	3.22	0.24	0.0575
18.35	2.34	2.42	−0.08	0.0064	2.29	0.05	0.0025	2.5	−0.16	0.0256
26.60	2.1	2.14	−0.04	0.0016	1.75	0.35	0.1222	1.98	0.12	0.0144
40.40	1.34	2.09	−0.75	0.562	1.35	−0.01	0.0001	1.58	−0.24	0.0576
				$\Sigma r^2=1.0076$			$\Sigma r^2=0.3901$			$\Sigma r^2=0.2946$
		$k=50$ m sec, $\varphi=-\pi/5.25$			$k=34.4$ m sec.			$a=0.81$, $b=31$.		

TABLE III.

Strength-duration-relation at the human retina in the light adaptation (950 lux.)

Duration in m sec	Threshold in volts									
	Obs.	Motokawa's theory			Hill's theory			Weiss' formula		
		Cal.	r	r^2	Cal.	r	r^2	Cal.	r	r^2
3.24	12.3	12.35	−0.05	0.0025	11.4	0.9	0.81	10.39	1.91	3.65
4.62	9.02	9.02	0	0	8.5	0.52	0.27	7.94	1.08	1.162
7.37	5.92	5.95	−0.03	0.0009	6.01	−0.09	0.0081	5.76	0.16	0.0256
10.1	4.87	4.66	0.21	0.044	4.93	−0.06	0.0036	4.83	0.04	0.0016
12.85	4	4	0	0	4.32	−0.32	0.1021	4.27	−0.27	0.0729
18.35	3.57	3.4	0.13	0.017	3.72	−0.15	0.0225	3.65	−0.08	0.0064
26.60	3.28	3.2	0.08	0.0064	3.35	−0.07	0.0049	3.12	0.16	0.0256
40.40	3.13	3.14	−0.01	0.0001	3.16	−0.03	0.0009	2.87	0.26	0.0676
				$\Sigma r^2=0.0709$			$\Sigma r^2=1.2221$			$\Sigma r^2=5.0117$
		$k=20$ m sec, $\varphi=-\pi/7$			$k=9.5$ m sec.			$a=2.21$, $b=26.5$		

TABLE IV.

Strength-duration-relation at the human retina in the complete dark adaptation.

Duration in m sec	Threshold in volts									
	Obs.	Motokawa's theory			Hill's theory			Weiss' formula		
		Cal.	r	r^2	Cal.	r	r^2	Cal.	r	r^2
4.62	11.7	12.2	–0.5	0.25	11.9	–0.2	0.04	10.47	1.23	1.513
7.37	8.19	8.03	0.16	0.0256	8.15	0.04	0.016	7.75	0.44	0.1936
10.1	6	6.2	–0.2	0.04	6.44	–0.44	0.1936	6.28	–0.28	0.0784
12.85	5.3	5.23	0.07	0.0049	5.47	–0.17	0.0289	5.43	–0.13	0.069
18.35	4.39	4.33	0.06	0.0036	4.45	–0.06	0.0036	4.5	–0.11	0.0121
26.6	3.92	4	–0.08	0.0064	3.77	0.15	0.0225	3.82	0.1	0.01
40.4	3.34	3.91	–0.57	0.325	3.34	0	0	3.3	0.04	0.0016
		$\Sigma r^2=0.6555$			$\Sigma r^2=0.3046$			$\Sigma r^2=1.8777$		
		$k=30$ m sec, $\varphi=-\pi/7$			$k=14.4$ m sec			$a=2.32$, $b=40$		

TABLE V.

Important measures for the electrical excitability of the human retina in various adaptation states.

Adaptation	Flatness R–curve	Amplitude of the 1. oscillation of E–curve	Damping coefficient	Rheobase in volts	Chronaxie in m sec
Adapted to 1 lux	1.37	120	1/50	0.84	30.8
Dark-adapted	1.69	30	1/30	2.49	13.8
Adapted to 950 lux	2.39	30	1/20	2.32	6.52

to some extent, it must be tested whether or not the agreement is specific to the theory in question. For reference, Hill's theory for the case of infinitely great λ: $I=\frac{I_o}{1-e^{-t/k}}$, (k: const.) and the well-known Weiss' experimental formula: $I=a+b/t$, (a, b: const.) were tested with the same data, and the calculated values are shown in the same tables. For the sake of comparison of fitness, the sum of squares of differences between observed and calculated values for each theory is given. The figures indicate that the best fitness was attained with Weiss' formula for the data in Table II, with Motokawa's theory for the data in Tab. III and with Hill's theory for the data in Tab. IV. Therefore, it is difficult to show the superiority of the theory for periodic systems merely on the basis of its fitness for the strength-duration-relation, but we can find a ground to take it as superior to any other theory in the following relations: 1. the value of k deter-

mined by this relation coincides with that obtained experimentally from the E-curves, the former being 50 m sec and the latter 49.1 m sec on an average for moderately light-adapted eyes. 2. the value of k is greatest for this adaptation state, and this means the slightest damping of the E-curves, because k is the reciprocal of the damping coefficient, just as has been actually the case with the experimental E-curves.

According to Hill's theory there must be a liner relation between the time-constant k and chronaxie τ, namely: $\tau=0.693\ k$. Though such a simple relation, of course, cannot be expected for periodic systems, it can be shown theoretically that the relation is not qualitatively altered. In Fig. 2 theoretical strength-duration-curves are shown, which were drawn with different values of k, but with the same values of the other parameters. As the ordinate of these curves is given in I/I_0, the chronaxie can be read as the abscissa for the point of intersection of the curves with the horizontal line $I/I_0=2$. From this Fig. it is seen that the chronaxie increases much more rapidly than in proportion to k. As an extreme case of increasing k or chronaxie we would have an automatic periodic excitation, because an infinitely great k theoretically corresponds to an undamped oscillation.

We have so far discussed the relation between periodic excitability and other physiological measures such as the accommodation constant λ, the rheobase, the time-constant k, the flatness of R-curves etc.,

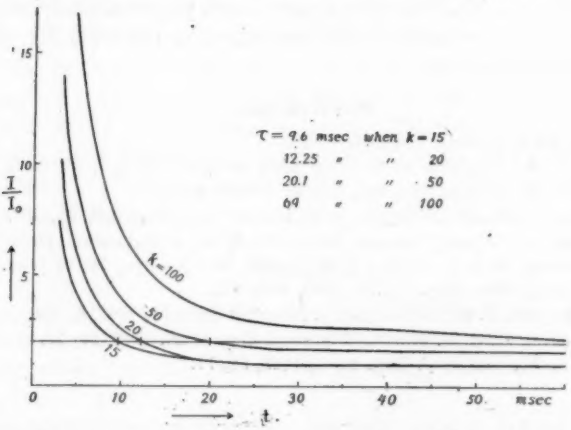


Fig. 2. Theoretical strength- duration- curves for periodic systems. Only k was varied without varying the other parameters. As I/I_0 is taken as ordinate the chronaxie equals the abscissa corresponding to the point of intersection of each curve with the horizontal line $I/I_0=2$.

and elucidated how important these factors are for the distinction of excitation types. But it is apparent from the above discussion that none of them as such can be a measure sufficient to express all the features of excitability.

Conclusion.

Excitable tissues may be classified into periodic, quasi-periodic and non-periodic systems. There are the following differences between periodic and non-periodic systems:

	Periodic systems	Non-periodic systems
(1)	Automatic excitation or multiple response to a single stimulus.	One response to a single stimulus.
(2)	Low rheobase.	High rheobase.
(3)	Large chronaxie.	Small chronaxie.
(4)	Large time-constant k.	Small k.
(5)	Slow accommodation.	Rapid accommodation.
(6)	Periodic E-curves.	Non-periodic E-curves.
(7)	True resonance.	Pararesonance.

The quasi-periodic system is a connecting link between periodic and non-periodic systems, and possesses the following properties: 1. it gives a single response to a brief stimulus, but multiple response to constant current. 2. it shows intermediate properties with regard to (2), (3), (4) and (5), and the same behavior as the non-periodic system with respect to (6) and (7). The above mentioned properties were theoretically elucidated on the basis of the experiments afforded by the author and other investigators.

References.

1) Katz, B., J. Physiol., 1937, **88**, 239.
2) Fessard, A., Propriétés rhythmiques de la matière vivante I, II, Paris, 1936.
3) Suzuki, M., Jap. J. Med. Sci. Biophys., 1943, **9**, 51.
4) Monnier, A.M. & G. Coppée, Arch. internat. physiol., 1939, **48**, 129.
5) Coppée, G., Symposia on quant. biol., 1936, **4**, 150. Arch. internat. physiol., 193, **40**, 1.
6) Motokawa, K. & K. Iwama, Jap. J. Med. Sci. Biophys., **11**, in press.
7) Hill, A.V., Proc. Roy. Soc. B, 1936, **119**, 305.
8) Kurebayashi, Y. & Y. Oku, Jap. J. Med. Sci. Biophys., 1943, **8**, 104.
9) Motokawa, K. & K. Iwama, Tohoku J. Exp. Med., 1949, **50**, 25.
10) Iwama, K., Tohoku J. Exp. Med., 1949, **50**, 71.

The Tohoku Journal of Experimental Medicine, Vol. 50, Nos. 3 & 4, 1949.

The Electric Excitability of the Human Eye as a Sensitive Indicator of Oxygen Deficiency.

By

Koiti Motokawa and Kituya Iwama.
(本川 弘一) (岩間 吉也)

(From the Physiological Laboratory of Prof. K. Motokawa, Tohoku University, Sendai.)

(Received for publication, September 6, 1946)

Dying patients often lose their visual sense earlier than any other senses and consciousness, and visual acuity becomes lessened at high altitude. The direct cause for these phenomena is lack of oxygen, as evidenced by a prompt recovery of visual function on administration of oxygen.

On account of such sensitivity to oxygen deficiency, the changes in visual function can, in turn, be used as an indicator of oxygen deficiency. The eye is most sensitive to light in its completely dark-adapted state, and this is the most suitable condition to prove any change in light-sensitivity. Therefore, it is necessary to perform the test of light-perception after the complete dark adaptation has been established, in order to detect slight oxygen deficiency with the decrease in light-sensitivity as an indicator. This is, however, inconvenient especially when other sorts of measurement have to be performed simultaneously or when the subject must be watched in order to give warning of his falling unconscious.

We have found the electric excitability of the eye to be no less sensitive to oxygen deficiency and more suitable for practical purposes. The method and its practical applications will be described in the following.

Method.

It has long been recognized among physiologists and psychologists that light-sensation is produced by electric excitation of the eye; a review of previous works on this subject is given in Rohracher's communication[1].

This author reported that the threshold took a minimum value at a frequency of about 20 cycles per sec. or Hz, when the eyes were stimulated with alternating currents of various frequencies. It has been found in

our previous experiments[2)3)] that the frequency corresponding to the threshold minimum was always a little lower than 20 Hz, and that the result was little altered when a periodically interrupted direct current was used as stimulating current instead of an alternating current with the same frequency as the interrupted one. This discovery has greatly simplified the production of stimulating currents in such experiments, because it is difficult to generate alternating currents with such low frequencies.

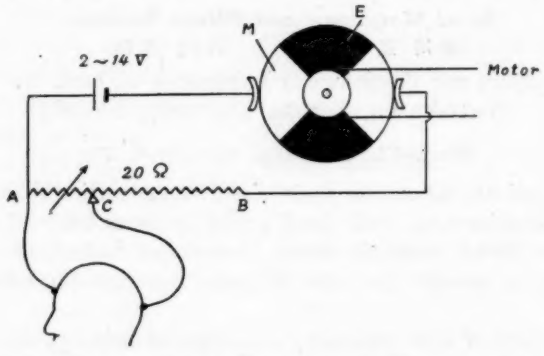


Fig. 1. Arrangement for electric stimulation of the human eyes. ABC: Voltage-divider. E: Ebonite-sector of the drum for interruption of direct current. M: Metallic sector.

Stimulating Currents.

The arrangement illustrated in Fig. 1 was used for the periodic interruption of the current. A drum consisting of metallic and ebonite-sectors of equal angle arranged alternately was driven by a motor, the speed of rotation of the drum being controlled by means of a system of pulleys and a resistance to the motor and measured accurately by means of a tachometer attached to the drum. The number of breakes per sec. or frequency was calculated from the speed of rotation and the number of sectors of the drum.

As mentioned above, the absolute value of threshold depends upon the frequency of the current, but its influence must be eliminated in our experiment to avoid confusion with the changes due to oxygen deficiency. In order to keep the frequency as constant as possible we tried to keep the voltage for the motor constant by means of an automatic regulator of voltage.

We made our subjects select the frequency most favorable for the determination of threshold, and worked with this frequency throughout the experiment, for the sensitivity of threshold to oxygen deficiency seemed to depend little on the frequency.

Electrodes.

Electrodes used were silver plates of 2×3 cm^2 in size and placed always upon the forehead (glabella) and the occiput, the electric contact being made with electrode-paste rich in NaCl. The current was passed always in such a direction as it flowed within the head from the occiput to the forehead, for the threshold for the current in this direction was lower than that in the reversed direction.

The chlorization of electrodes for the purpose of making them non-polarizable was found to be of little use; the large area of electrodes and good electric contact were of great advantage for this purpose.

Determination of Threshold.

The voltage of the stimulating current was varied between 0 and 14 volts by means of a voltage-divider which could be operated very smoothly. When the voltage was gradually raised the subject began to feel flickering synchronous with the pulses of the stimulating current at a certain value of voltage. This is the threshold for appearance and denoted by Va. The threshold for disappearance (Vd) is the critical voltage at which flickering disappears, when the voltage is gradually decreased from its value sufficiently high to produce a distinct flicker.

We were used to inform our subject of the commencement of the test in some way, say, by ringing a bell, in order to direct his attention to the appearing or disappearing flicker. The speed of increase or decrease of voltage, which was manually regulated by the investigator, influenced the absolute value of threshold; but it was no great concern in our experiment, in which only relative values were important, so that it sufficed to keept the speed as constant as possible. However, the easiness with which the threshold was determined depended greatly upon the speed of voltage increase or decrease, and the most favorable speed was somewhat different from subject to subject, so that it had to be selected by each subject.

The threshold for disappearance depended to some extent upon the level to which the voltage had previously been raised; in our experiment it was raised until the subject felt a very distinct flicker and yet no disagreeable sensation due to excitation of other sensory receptors. This voltage was in most cases about 10 volts. It seemed that some simple mechanical device would serve to keep the speed of voltage-change constant, but this has not yet been realized, for it has proved to be no simple matter to meet all the requirements metnioned above.

Illumination and Adaptation State of the Eye.

One of the merits of our method consists in the greatest accuracy of measurement being attained not in complete darkness, but at moderate

illumination; a constant illumination of 1–50 lux seemed to be most adequate. Subjects who had stayed long in the open air had to wait until their eyes became adapted to the illumination in the test-room, for otherwise the threshold would decrease with the proceeding of adaptation and lead to erroneous results.

The eyes had to be fixed by means of a fixation-point during the stimulation, because the threshold could be lowered by eye-movements.

Results and Discussion.

1. *Low Atmospheric Pressure and the Electric Excitability of the Eye.*

The experiment was carried out in a large low pressure chamber, the inside of which was dimly lighted. The subject was seated before the wall of the chamber and gazed at a point marked on it. As soon as he recognized the appearance or disappearance of flicker he pressed a button to inform the investigator outside the chamber of his recognition of it. Upon this information the investigator stopped moving the slide-contact of the voltage-divider and read the value of threshold on the scales attached to the voltage-divider.

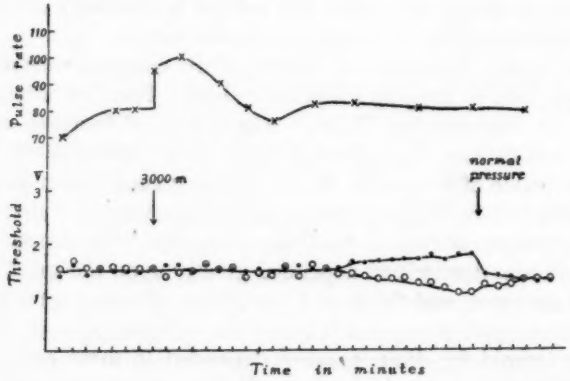


Fig. 2. Effect of low atmospheric pressure equivalent to that at 3,000 m altitude on the electric excitability of the eye and the pulse rate. Solid circles: Threshold for appearance. Empty circles: Threshold for disappearance.

The time taken for a single measurement, i.e. the time from the beginning of increase or decrease of voltage to the reading of the threshold was about 2 seconds. The two sorts of threshold, namely Va and Vd were obtained successively without any interval. An example of our results obtained from five medical students is shown in Fig. 2. As seen from this Fig., Va (expressed by solid circles) was somewhat higher than Vd (expressed by empty circles) in all cases investigated. The difference between

Va and Vd was generally greater at subjects who were not yet accustomed to such measurements, and decreased to some extent by exercises of measurement.

The atmospheric pressure in the chamber was lowered at a rate of about 500 m per minute until it became equivalent to the pressure at 3,000 m (9,830 feet) altitude, and hereafter maintained at this level. The threshold remained at first unaltered, but began to change in about 14 minutes in such a manner that the difference between Va and Vd increased with the lapse of time. This increase in difference was caused apparently by the rise in Va and at the same time by the lowering of Vd so that the mean value of Va and Vd remained unaltered.

If one takes, as usual, the mean value as threshold one must say that the excitability of the eye suffers no alteration at all by the action of the low atmospheric pressure equivalent to that at 3,000 m altitude, but if the difference between Va and Vd is taken as an index, a remarkable effect of the low atmospheric pressure can be seen, as illustrated in Fig. 2. The increasing divergence of Va and Vd from each other is surely the effect of low exygen pressure, for the difference decreases up to the normal magnitude by administration of oxygen or by raising the atmospheric pressure to normal level.

Now the question arises why the effect of exygen deficiency shows itself in the difference of Va and Vd, but not in their mean value. This relation may find its explanation in the circumstances that the two measures are concerned with different aspects of electric excitability which do not always run parallel. We know that electric excitability may be analysed into two factors, namely intensity- and time factor; for instance, in excitation experiments with constant currents the rheobase is the intensity factor, and the chronaxie is the time factor.

Our thresholds Va and Vd imply not only the intensity factor, but also the time factor. This is apparent from the manner of threshold determination in our experiment, in which the investigator continues to raise or lower voltage, until he receives the signal of recognition from the subject. If the reaction-time of the subject is prolonged as the result of oxygen deficiency, Va must rise, but Vd may be lowered, so that the diffrence between them consequently increases. Hence, the difference expresses not so much the intensity factor as the time factor of the electric excitability of the human eye. On the contrary the mean value of Va and Vd expresses the intensity factor alone, because the errors due to the reaction-time cancel out each other in the mean.

If this argument is correct the above fact must be so interpreted that the time factor is more sensitive to oxygen deficiency than the intensity factor. However, it is still open to question whether the seat of increase

in the reaction-time is acutually the retina itself or whether other organs and tissues are involved in it such as the optic nerve, the brain, the motor nerve and the muscle. In order to answer this question, we need only examine the effect of oxygen deficiency on the reaction-time for any other sort of stimulus. If the reaction-time for, say, the skin-sensation is not prolonged by the action of oxygen deficiency, we can safely conclude that the retina is responsible for the increase in the reaction-time.

In fact, we measured the electric threshold for skin-sensation with the same method of stimulation as in the above experiment instead of measurements of the reaction-time itself, because it was more convenient for the comparison of both sorts of results. In this experiment Va was somewhat higher than Vd, just like in the above experiment; but the absolute value of threshold was in general much higher than that for the eye. It must be emphasized that the difference between Va and Vd showed no increase at all even at such a high altitude as 5,000 m (16,380 feet), provided that the stay at that atmospheric pressure was not too long (within 20 minutes). Thus the special sensitivity of the retina to oxygen deficiency has been confirmed.

It is interesting to compare the behavior of the pulse rate with that of the electric excitability; in the case illustrated in Fig. 2, the pulse rate increased rapidly on exposure to low atmospheric pressure and then decreased gradually to the normal level, while no change in electric excitability was seen yet at this stage. The initial increase in the pulse rate is probably due to some mental excitement caused by the procedure of air-withdrawal and other factors, for otherwise the subsequent decrease and the want of association with any change in electric excitability will be difficult to explain.

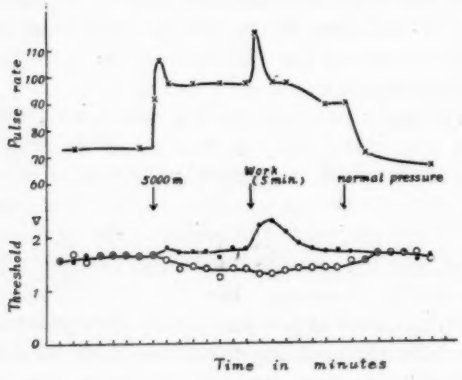


Fig. 3. Effect of low atmospheric pressure equivalent to that at 5,000 m altitude. Solid circles: Threshold for appearance. Empty circles: Threshold for disappearance.

Fig. 3 shows the result obtained from the same subject, the atomospheric pressure being equivalent to that at 5,000 m (16,380 feet) altitude. The divergence of Va and Vd from each other set in, as soon as the low pressure was imposed, and increased gradually with the lapse of time. In order to see the effect of muscular

work on the electric excitability under these conditions, we made our subject work for five minutes, lifting and lowering successively a weight of about 30 kg. The divergence of thresholds increased markedly at the beginning of the work, but began to decrease already during the working period. It must be noted that this change in electric excitability was associated with a similar change in the pulse rate. We have interpreted the initial rise in pulse rate at 3,000 m altitude in the former case as the result of some mental excitement, because there was no corresponding change in electric excitability. On the contrary, the metabolic changes due to muscular work must lead to parallel changes in electric excitability and pulse rate, as has been found in the present experiment, because such changes as the increase in carbonic acid in the blood will raise the pulse rate, and the decrease in oxygen concentration will change the electric excitability.

It is seen from Fig. 3 that the increase of difference between Va and Vd caused by muscular work has been brought about by further rise of Va, but that Vd has contributed nothing to it. The mean threshold rose consequently as the result of muscular work. It seems that greater oxygen requirement in muscular work must have increased its deficiency to such an extent as to affect the less sensitive intensity factor of electric excitability of the eye.

2. *Parallel Experiments on the Light-Sensitivity and the Electric Excitability of the Human Eye under Low Oxygen Pressure.*

We performed simultaneous measurements of light-sensitivity and electric excitability in the low pressure chamber to answer the question,

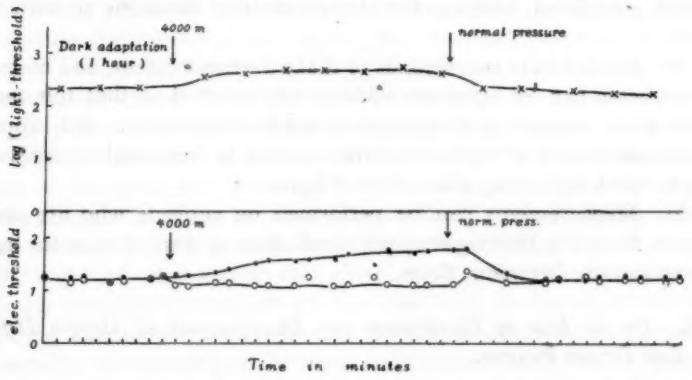


Fig. 4. Comparison of the effect of low oxygen pressure on light-sensitivity with that on the electric excitability of the human eyes. Measurements were performed in the dark after dark adaptation had been established.

which of them was more sensitive to oxygen deficiency. For determination of light-thresholds Nagel's adaptometer was used. The experiment was carried out in complete darkness after the light-threshold had become constant by an hour's dark adaptation. The light-threshold was measured every 3 minutes, and the electric threshold every minute.

The result obtained from another subject at 4,000 m (13,110 feet) altitude is shown in Fig. 4, in which the light-threshold is given in logarithmic scales. On exposure to the low pressure the light-threshold rose slightly and remained almost constant until it began to decrease by removal of the low pressure. This behavior is so similar to that of the electric threshold that there is no doubt about the essential relationship between both phenomena. The relative merits of the two as the index of oxygen deficiency should be determined by comparing their accuracy as well as utility with each other. After a careful survey we have reached the conclusion that the electric method is superior to the method of light-sensitivity, especially in the following points:

(1) Greater accuracy of measurement is more easily attainable by the electric method. This, however, does not mean that the accuracy of measurement is always higher in the electric method than in the other; in fact, the measurement of light-sensitivity can be as accurate as that of electric excitability under the most favorable conditions, but such conditions are, for the former, much more difficult to realize.

(2) The electric method is advantageous especially, when simultaneous measurements of other kinds are needed, for it can be applied to experiments in a lighted room.

(3) In the test of light-sensitivity we must wait until dark adaptation has been completed, whereas the electric method demands no such waste of time.

(4) It takes only two seconds to make a measurement, and moreover, measurements can be repeated without any interval so that this method enables us to pursue rapid changes in oxygen deficiency. It is apparent that measurements of light-sensitivity cannot be repeated so frequently, owing to the long lasting after-effect of light.

(5) Measurements can be performed on subjects who are situated far apart from the investigator, provided there is some device for mutual communication between them.

3. *On the Rate of Development and Disappearance of Oxygen Deficiency due to Low Oxygen Pressure.*

The rapidity with which the electric threshold can be measured allows us to follow the rapid development of oxygen deficiency at as low atmospheric pressure as equivalent to that at 7,000–8,000 m altitude. The

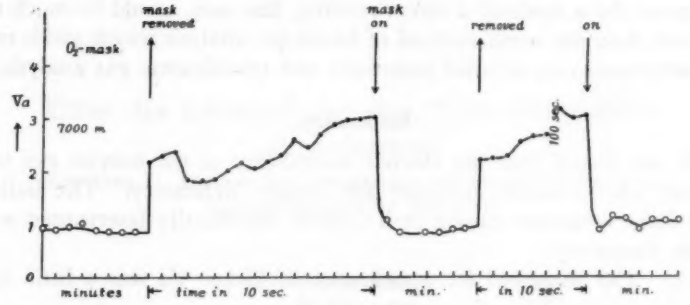


Fig. 5. Course of development and disappearance of oxygen deficiency as measured with thresholds for appearance of flicker as the index.

rise in threshold is usually so marked that we need no more rely upon the difference between Va and Vd, and may omit measurement of Vd. An example of such experiments is illustrated in Fig. 5 in which the values of Va measured every minute are expressed by empty circles and those measured every 10 seconds by solid circles. Even at a 7,000 m (22,950 feet) altitude the threshold remained normal, when an oxygen mask was used, but it rose promptly to a level over two times as high as normal, when the subject was exposed to the low pressure by removing the oxygen mask. This change must have taken place in less than 2 seconds, for the rise is clearly seen in the first value of Va which was determined 2 seconds after the onset of low pressure.

It is interesting to note that after this abrupt initial rise the threshold increased only gradually, showing in some cases a slight temporary decrease. On wearing the oxygen mask again, the threshold returned to normal level with the same rapidity as at the initial rise. The course of these changes reminds us of the oxygen concentration in the arterial blood. It is hardly possible to follow exactly the change in oxygen concentration in the blood within such a short period as that of the initial rise, and it is difficult to compare the course of both phenomena, but very plausible that the change in oxygen concentration in the blood is faithfully reflected in the course of electric excitability. The abrupt initial rise of threshold may be regarded as evidence that there is no oxygen depot whatever in our body, and the subsequent gradual rise may be indicative of slowly developing decompensation. At any rate, it is desirable to ground experimentally the parallelism between our measure and the oxygen concentration in the arterial blood, how difficult a task it may be.

If complete parallelism or some quantitative relation had been established between them, the utility of our method would become consider-

ably great, for a method of direct reading, like ours, would be much more practical than the usual method of blood-gas analysis which yields results only after annoying arterial punctures and troublesome gas analysis.

Summary.

It was found that the electric excitability of the human eye was a sensitive and accurate measure for oxygen deficiency. The indicator was a flicker-sensation caused by a current periodically interrupted with a certain frequency.

(1) The threshold for appearance of flicker Va was a little higher than the threshold for disappearance Vd.

(2) The difference between Va and Vd increased always at low oxygen pressure equivalent to that at 3,000 m or higher altitude, but the mean value of Va and Vd increased only at severer oxygen deficiency.

(3) This method is suitable for following rapid changes of oxygen deficiency, for it takes only 2 seconds to determine the threshold.

(4) The electric excitability of the eye was studied in comparison with its sensitivity to light, and found to be more suitable as an index of oxygen deficiency for accurate measurements and practical applications than the light-sensitivity of the eye.

References.

1) Rohracher, H., Z. f. Sinnesphysiol., 1935, **66**, 164.
2) Motokawa, K. & K. Iwama, Japanese J. Med. Sci. Biophy., **11**, in press.
3) Motokawa, K. & K. Iwama, Tohoku J. Exp. Med., 1949, **50**, 307.

The Tohoku Journal of Experimental Medicine, Vol. 50, Nos. 3 & 4, 1949.

Über die Lebensdauer der Tuberkelbazillen in Exkreten.

Mitteilung I: Einfluss der Temperatur auf die Lebensdauer der Tuberkelbazillen in Exkreten.

Von

Takesi Abe.
(阿部 武)

(Aus der Medizinischen Klinik und dem Kosankinbyo Kenkyusyo der Kaiserlichen Tohoku-Universität zu Sendai. Leiter: Prof. Der. T. Kumagai und Prof. Dr. T. Ebina.)

(Received for publication, April 4, 1945)

1. Einleitung.

Um den Infektionsprozess der Tuberkulose zu besprechen und geeignete Vorbeugungsmassregeln gegen die Infektion zu treffen, muss man selbstverständlich die Lebensdauer der Tuberkelbazillen, welche mit den Ausscheidungsmaterialien zusammen aus dem Körper des Patienten in die Aussenwelt ausgeschieden worden sind, unter verschiedenen Bedingungen klar machen. Darum wurden seit langem von vielen Autoren, Koch[1] an der Spitze, viele Untersuchungen darüber gemacht. Allein ihre Ergebnisse sind sehr verschieden wie ihre Versuchsmethoden und die Bedingungen, unter denen sie ihre Versuche anstellten. Besonders selten sind solche Untersuchungen, in denen man Lebensdauer der Tuberkelbazillen nach den Jahreszeiten systematisch studierte und verglich. Das Versuchsmaterial ist vorwiegend auf Sputum und Bazillenemulsion beschränkt. Mit Kot und Harn haben wohl Ito,[2] Ogawa,[3] Hasegawa[4] u.a. Versuche angestellt, aber es scheint doch kein Versuch mit Eiter gemacht worden zu sein. Da wir den Zusammenhang zwischen der Lebensdauer der Tuberkelbazillen in allen Ausscheidungsmaterialien, Sputum, Harn, Kot und Eiter, und der Temperatur, die unter den Bedingungen der Aussenwelt besonders wichtig ist, durch die vier Jahreszeiten hindurch untersucht haben, seien die Ergebnisse im folgenden mitgeteilt.

2. Versuchsmethode und -material.

Zum Versuche wurden Sputum, Harn, Kot und Eiter von den in unserer Klinik aufgenommenen Patienten von offener Lungen-, Nieren-,

Darmtuberkulose und Pyothorax in der Tagesmenge verwendet. Nachdem die Versuchsmaterialien hinreichend umgerührt und möglichst homogen gemacht worden sind, wurden je 1 ccm, 20 ccm, daumenspitzgrosses Klümpchen bzw. 1 ccm. von ihnen auf Schalen mit Decke oder Reagenzgläser mit Wattepfropf verteilt und in einer Tieftemperaturkammer bei 2–3° C, unter dem Tische im Wohnzimmer, im dunkelen Wandschrank des Wohnzimmers und im Thermostat bei 37° C aufbewahrt. In bestimmten Zeiträumen wurden sie 10 Minuten lang mit 20 ccm 4% H_2SO^4 behandelt und dann wieder 10 Minuten lang mit 3000 Drehungen pro Minute zentrifugiert. Mit dem Bodensatz wurde die Kultur auf 2–3 Oka-Katakura schen Nährboden ausgeführt. Durch 3 Monate lange Beobachtung wurde festgestellt, ob die Bazillen leben oder gestorben sind. Andererseits wurde eine Öse Sputum auf den Agarnährboden gestrichen, um die Wucherung verschiedener Bakterien zu prüfen. Dann wurde der Zusammenhang zwischen diesen verschiedenen Bedingungen der Aussenwelt und der Lebensdauer der Tuberkelbazillen in den Ausscheidungsmaterialien untersucht.

3. Versuchsresultate.

1) Lebensdauer der Tuberkelbazillen in den Exkreten, die der Tieftemperaturkammer aufbewahrt sind. Was die Lebensdauer der Tuberkelbazillen in den verschiedenen Ausscheidungsmaterialen, welche im Zimmer bei 2–3° C aufbewahrt sind, anbelangt, ist sie, wenn man ebenso wie in den folgenden Versuchen den positiven Ausfall der Kultur als lebend, den negativen als tot bezeichnet, wie folgt:

Sputum: Bei der Aufbewahrung in der Schale mit Decke beträgt die Lebensdauer der Tuberkelbazillen durchschnittlich 192 Tage, also ungefähr 6 Monate. Bis zum Aussterben brauchen sie 9 Monate. Im Reagenzglas mit Wattepfropf beträgt sie 237 Tage, d.h. ungefähr 8 Monate bzw. 9.5 Monate.

Harn: Im Reagenzglas leben die Tuberkelbazillen durchschnittlich 5 Monate lang und sterben in 9 Monaten aus.

Kot: Im Reagenzglas leben sie durchschnittlich nur 2 Wochen lang und sterben in 4 Wochen aus.

Eiter: Im Reagenzglas leben sie 11 Monate lang und sterben in einem Jahre aus. Die Kolonien nehmen sehr langsam ab und verschwinden endlich.

2) Lebensdauer der Tuberkelbazillen in den Ausscheidungsmaterialien, die an dunklen Orten im Wohnzimmer aufbewahrt sind.

Wenn die Ausscheidungsmaterialien im Dunkeln unter dem Tische im Wohnzimmer, dessen Glasfenster nach Norden und Westen gehen, und im dunkeln Wandschrank des Wohnzimmers aufbewahrt sind, so

Tabelle I.

Zusammenhang Zwischen der Lebensdauer der Tuberkelbazillen in Exkreten und Temperatur.

Ort.	Ausscheidungs-substanz	Behältnis u.a.		Jahreszeit	Lebensdauer	bis zum Sterben
Tieftemperatur-kammer (2–3°C)	Sputum	Schale			192 Tage	270 Tage
		Reagenzglas			237 "	285 "
	Harn	Reagenzglas			156 "	235 "
	Kot	Reagenzglas			14 "	31 "
	Eiter	Reagenzglas			333 "	374 "
Wohnzimmer	Sputum	Schale	unter dem Tische	Frühling	77 "	100 "
				Sommer	37 "	52 "
				Herbst	89 "	120 "
				Winter	78 "	95 "
			in dunkelen Wandschrank	Frühling	100 "	120 "
				Sommer	42 "	63 "
				Herbst	120 "	150 "
				Winter	120 "	150 "
	Harn	Reagenzglas	unter dem Tische	Winter	72 "	86 "
	Kot	Reagenzglas	unter dem Tische	Sommer	0 "	1 "
				Herbst	3 "	4 "
				Winter	9 "	11 "
	Eiter	Schale	unter dem Tische	Sommer	31 "	56 "
Thermostat (37°C)	Sputum	Schale			8 "	10 "
		Reagenzglas			8 "	10 "
	Harn	Reagenzglas			9 "	12 "
	Kot	Reagenzglas			0 "	1 "
	Eiter	Reagenzglas			9 "	10 "

TABELLE II.

Wucherungszustand Verschiedener Bakterien im Sputum an Verschiedenen Aufbewahrungsorten.

Ort	Behältnis	Kranke \ Resultat / Aufbewahrungstage	0	1	2	3	4	5	6	7	8	9	10	14	19	21	24	31	41	50	89	101	die Zahl der Tage, bis sie viel werden	
			Kolonien der verschiedenen Bakterien																					
Tieftemperaturkammer	Schale	Yamaguti S.	±						+				+	+			+				++		89	
		Yamaguti T.	±	+	+	−	−	+	+	+			+					+	+			++	101	93
	Reagenzglas	Sato T.	±						+		+		−	−			+				++		89	
Wohnungszimmer	Schale	Yamaguti S.	+		+			+		+			++		|||							10	
		Yamaguti T.	+	+	++	++	++	++		++							++	|||	|||			24	15
	Reagenzglas	Sato T.	+		+			+		+			++		++	++							10	
Thermostat	Schale	Yamaguti S.	+	|||	|||	|||	|||	|||	|||			++			+						1	
		Sato T.	+	|||	|||	|||	|||		|||		|||	|||			++	−				1	1
		Yamaguti T.	+	|||	|||	|||	|||	|||	|||			++	+		−						1	
		Aratani M.	+	|||	|||	|||	++	++	−	−		−	−				+	−				1	

Anmerkungen: ± 0∼5
+ 6∼15
++ 16∼25
||| 26∼50
|||| 51∼

beträgt die Lebensdauer der Tuberkelbazillen wie folgt.

Sputum: Wenn das Sputum in einer Schale unter dem Tische aufbewahrt ist, so leben die Tuberkelbazillen vom Frühling bis zum Sommer 77 Tage, im Sommer 37 Tage, vom Herbst bis zum Winter 89 Tage und vom Winter bis zum Frühling 78 Tage lang und sie sterben in 100, 53, 120 bzw. 95 Tagen aus.

In dunkelen Wandschrank leben sie vom Frühling bis zum Sommer 100, im Sommer 42, vom Herbst bis zum Winter 120 und vom Winter bis zum Frühling ebenfalls 120 Tage lang, und sterben in 120, 63, 150 bzw. 150 Tagen aus.

Harn: Im Reagenzglas unter dem Tische leben sie im Winter durchschnittlich 72 Tage lang und sterben in 89 Tagen aus.

Kot: Unter dem Tische aufbewahrt, können die Tuberkelbazillen im Sommer schon nach einem Tage nicht mehr nachgewiesen werden, während sie im Herbst bis zum 3 und im Winter bis zum 9 Tage nachgewiesen werden.

3) Lebensdauer der Tuberkelbazillen in den Ausscheidungsmaterialien, die in dem Thermostat bei 37°C aufbewahrt sind.

Sputum: Unabhängig von der Art des Behälters leben die Tuberkelbazillen durchschnittlich 8 Tage lang und sterben in 10 Tagen aus.

Harn: Ähnlich wie im Sputum leben die Tuberkelbazillen durchschnittlich 9 Tage lang und sterben in 13 Tagen aus.

Eiter: Es sind gleiche Resultate wie bei Harn erzielt.

Kot: In einem Tage können die Bazillen nicht mehr nachgewiesen werden.

4) Wucherungszustand verschiedener Bakterien im Sputum an verschiedenen Aufbewahrungsorten. Wenn das Sputum an den oben erwähnten verschiedenen Orten stehen gleassen wird, so wuchern verschiedene Bakterien im Zimmer bei einer niederen Temperatur von 2–3°C so geringfügig und langsam, dass sie im grossen und ganzen erst nach 3 Monaten viel werden. Bei 37°C dagegen werden sie schon in einem Tage zahllos. Im Wohnzimmer werden sie nach 15 Tagen verhältnismässig viel, darum steht das Wohnzimmer betreffs der Wucherung verschiedener Bakterien zwischen beiden oben genannten Orten. Sie sind hauptsächlich grampositive Staphylokokken, und manchmal sind Heubazillen und Pilze gemischt.

4. Zusammenfassung und Betrachtung.

1) Zusammenhang zwischen der Lebensdauer der Tuberkelbazillen in den Ausscheidungsmaterialien und der Zimmertemperatur. Honma und Koida[5] u.a. sagten, dass eine tiefe Temperatur auf die Tuberkelbazillen keine schädliche Wirkung hat. Nach Shope[6] sind die Tuberkelbazil-

len in einer Emulsion in der Eiskammer nach Ablauf von 310–330 Tagen endlich ausgestorben. Hasimoto[7] beobachtete ferner bei ungefähr 20°C während 30 Tage gar kein Wachstum der Tuberkelbazillen. Von vielen Autoren ist es jedoch erkannt worden, dass die Tuberkelbazillen bei Kälte lange leben können. Wenn auch Kirstein[8] sagte, dass die Tuberkelbazillen bei Zimmertemperatur innerhalb 40 Tage aussterben, behaupteten Schill und Fischer[9] und Sawitzky,[10] dass sie binnen 226 Tage aussterben. Ogawa[5] fand, dass die Tuberkelbazillen im Harn nach Ablauf von 18 Tagen noch leben. Im Kot können die Tuberkelbazillen nach Ogawa[3] und Ito[2] 8 Tage oder noch länger, nach Hasegawa[4] 50 Tage lang leben. Kuwahara und Uesaka[11] stellten fest, dass die Tuberkelbazillen bei 37°C aus Sputum nach 3 Tagen nicht mehr herausgezüchtet werden. Nach Richet und Handuroy[12] aber wird die Kultur aus Bazillenemulsion erst nach 10–15 Tagen negativ. Unsere Ergebnisse stimmen mit denen der Autoren darin überein, dass die Tuberkelbazillen im Zimmer bei einer tiefen Temperatur am längsten leben können, d.h. sie leben im Sputum 6–8 Monate, im Harn 5 Monate, im Eiter 11 Monate lang und im Kot länger als 2 Wochen. Dann folgen die im Wohnzimmer. Im dunkelen Wandschrank des Wohnzimmers bleiben manche Bazillen im Sputum gar 4 Monate lang am Leben. Auch unter dem Tische, wo nur spärliches Licht hinkommt, leben sie im Sputum 1,5–3 Monate, im Harn 2.5 Monate lang, im Kot weniger als 10 Tage und im Eiter länger als 1 Monat. Dagegen leben sie am kürzesten, wenn die Ausscheidungsmaterialien im Thermostat bei 37°C liegen, da sie nämlich im Sputum, Harn und Eiter durchschnittlich nur 8–9 Tag, wenn auch manche länger als 2 Wochen, leben. Im Kot sterben sie schon in enem Tage aus.

Wenn auch wohl komplizierte Gründe dazu vorhanden sind, dass die Lebensdauer der Tuberkelbazillen je nach der Termperatur an den Aufbewahrungsorten so verschieden ist, muss ihre Klärung für die Morphologie der Tuberkelbazillen von sehr grosser Bedeutung sein, von der Vorbeugung gegen die Tuberkulose ganz zu schweigen. Darum haben wir gewissermassen versucht auch dieses Problem zu verfolgen. Wie aus Tabelle 2 ersichtlich ist, geht die Wucherung verschiedener Bakterien im Sputum so vonstatten, dass sie im Zimmer bei einer tiefen Temperatur erst nach 3 Monaten viele Kolonien entwickeln, während sie bei 37°C in einem Tage zahllos und im Dunkeln ungefähr nach 15 Tagen viel werden. Bei 37°C gehen die Fäulnis und Gärung nämlich mit der Wucherung verschiedener Bakterien zusammen sehr schnell vor sich, wodurch das Leben der Tuberkelbazillen in hohem Gräde bedroht wird. Nach Ozeki[13] ist die Atmung der Tuberkelbazillen bei 37°C und 45°C am höchsten. Setzt man diese Atmung als 1/1, so beträgt

sie bei 20°, 12° und O° C 1/2–1/3, 1/4–1/3 bzw. 1/4–1/5. Mithin wird bei 37°C der Nährstoff am meisten in Anspruch genommen. Da es jedoch das Medium nicht gestattet, so sterben die Tuberkelbazillen unseres Erachtens schnell aus. Im Zimmer bei einer tiefen Temperatur sind die Eingriffe durch die Wucherung verschiedener Bakterien, Fuälnis, Gärung usw. nur gering und ist die Atmung der Tuberkelbazillen auch überdies sehr schwach. Folglich werden die Tuberkelbazillen den Nährstoff nicht einnehmen und im Scheinschlaf liegen; deshalb wird wahrscheinlich ihr Leben sehr lange dauern. Da im Wohnzimmer die Werte zwischen den beiden gewonnen sind, wird ihr Lebenedauer dementsprechend ziemlich lange sein. Dass die Tuberkelbazillen im Eiter im Zimmer bei einer tiefen Temperatur länger als 11 Monate leben können, ist wahrscheinlich darauf zurückzuführen, dass verschiedene Bazillen nur spärlich wuchern, da Eiter im aseptischen Verfahren entnommen ist. Dass die Tuberkelbazillen dagegen im Kot am schnellsten aussterben, kommt es vielleicht davon her, dass verschiedene Bakterien am erheblichsten wuchern.

2) Zusammenhang zwischen der Lebensdauer der Tuberkelbazillen im Wohnzimmer und den Jahreszeiten. Die Lebensdauer der Tuberkelbazillen in den Ausscheidungsmaterialien im Wohnzimmer ist bisher nur selten den Jahreszeiten nach untersucht und verglichen worden. Wir haben dies an 2 Stellen, unter dem Tische und im dunkelen Wandschrank des Wohnzimmers untersucht und festgestellt, dass die Lebensdauer der Tuberkelbazillen im Sommer am kürzesten, vom Frühling bis zum Sommer etwas länger, vom Winter bis zum Frühling noch länger und vom Herbst bis zum Winter am längsten ist; sie nimmt also in der Reihenfolge Sommer, Frühling, Herbst, Winter zu. Auch in den Versuchen mit Kot und Eiter unter dem Tische sind ähnliche Resultate erzielt worden. Die Gründe zu dieser Erscheinung bestehen wahrscheinlich darin, dass, wie im vorhergenden Abschnitt erwähnt ist, je nach der Differenz der Temperatur die Wucherung verschiedener Bakterien, die Fäulnis und Gärung der Ausscheindungsmaterialien und der Stoffwechsel der Tuberkelbazillen verschieden vor sich gehen. Daher wird die Gefahr der Tuberkuloseinfektion im Zimmer im Winter am grössten sein.

5. Schluss.

1) Was die Lebens-dauer der Tuberkelbazillen in den Ausscheidungsmaterialien anbelangt, wird im allgemeinen kein Unterschied zwischen Sputum und Harn beobachtet. Im Eiter leben die Bazillen im Vergleich mit den beiden wenn auch nur gering länger. Im Kot leben sie am kürzesten.

2) Die Tuberkelbazillen im Sputum, Harn und Eiter können 4

Monate lang leben, wenn diese im Dunkeln im Zimmer stehen gelassen werden. Auch an einem halbdunklen Orte können manche 3 Monate lang leben. Im Kot sterben sie dagegen in einigen Tagen aus.

3) Die Tuberkelbazillen im Zimmer leben im Sommer am kürzesten, im Fühling etwas länger und im Winter am längsten.

4) Während die Tuberkelbazillen im Sputum oder Harn bei 37°C in ungefähr 10 Tagen aussterben, leben sie im Zimmer bei einer tiefen Temperatur von 2–3°C 6–8 Monate lang.

Diese Forschung wurde durch Überlassung von Geldmitteln des Unterrichtsministeriums für wissenschaftliche Forschung ausgeführt, wofür herzlich gedankt sei. Prof. Dr. T. Kumagai und
Prof. Dr. T. Ebina.

Literatur.

1) Koch, R., Schwalbe: Gesammelte Werke von Robert Koch. Lpz., 1912, 656.
2) Ito, A. und Fujino, T., Tokyo Iji Ss., 1934, 2874, 932.
3) Ogawa, G., Kekkaku, 1938, **16**, 355.
4) Hasegawa, F., Kekkaku, 1936, **14**, 353.
5) Honma, E. und Koita, K., Manshu Ig. Z., 1927, **1**, 145.
6) Shope, R., J. of exper. Med., 1926, **44**, 623.
7) Hasimoto, T., Manshu Ig. Z., 1937, **26**, 1.
8) Kirstein, F., Z. Hyg., 1905, **50**, 186.
9) Schill, E. und Fischer, B., Mitt. ksl. Gesdh. Amt., 1884, **2**, 131.
10) Sawitzky. W., Zbl. Bakter., 1892, **11**, 153.
11) Kuwahara, T. und Uesaka, I., Kekkaku Iho., 1942, **6**, 369.
12) Richet, C. et Hunduroy, P., C. r. Soc. Biol. 1926, **95**, 555.
13) Ozeki, R., Tohoku J. Exp. Med., 1944, **46**, 222.

The Tohoku Journal of Experimental Medicine, Vol. 50, Nos. 3 & 4, 1949.

Über die Lebensdauer der Tuberkelbazillen in Exkreten.

Mitteilung II: Einfluss des Sonnenlichtes auf die Lebensdauer der Tuberkelbazillen in Exkreten.

Von

Takeşi Abe.
(阿 部 武)

(Aus der Medizinischen Klinik und dem Kosankinobyo Kenkyusyo der Kaiserlichen Tohoku-Universität zu Sendai. Leiter: Prof. Dr. T. Kumagai und Prof. Dr. T. Ebina.)

(Received for publication, April 14, 1945)

1. Einleitung.

Wir haben in der Mitteilung I den Zusammenhang zwischen der Lebensdauer der Tuberkelbazillen in den Ausscheidungsmaterialien und der Temperatur berichtet. Dass das Sonnenlicht auf die Tuberkelbazillen ebenso bedeutendsten Einfluss wie die Temperatur ausübt, ist von Koch[1] und vielen andern Autoren geklärt worden. Aber es gibt verhältnismässig wenig solche Angaben, in denen man diesen Zusammenhang den Jahreszeiten nach studiert und verglichen hat.

Wir haben in vorliegender Arbeit den Zusammenhang zwischen der Lebensdauer der Tuberkelbazillen in allen Ausscheidungsmaterialien und dem Sonnenlicht den Jahreszeiten nach untersucht. Die Ergebnisse seien im folgenden mitgeteilt.

2. Versuchsmethode und -material.

Wie in der vorhergehenden Mitteilung erwähnt ist, wurden Sputum, Harn, Kot und Eiter in Schalen mit oder ohne Decke oder Reagenzgläser mit Watte-pfropf verteilt und am Glasfenster des Wohnzimmers, im diffusen Licht im Freien und in der direkten Bestrahlung der Sonne auf dem Dache aufbewahrt. In bestimmten Zeiträumen wurden sie nach Behandlung mit 4% H_2SO_4 auf 2–3 Oka-Katakuraschen Nährboden kultiviert, und durch die 3 Monate lange Beobachtung wurde festgestellt, ob die Bazillen leben oder gestorben sind. Andererseits haben wir eine Öse davon auf das Nähragar ausgestrichen und den Wucherungszustand verschiedener Bazillen untersucht. Ausser der Temperatur, der Feuchtigkeit

und der Windkraft an Versuchstagen haben wir ferner mittelst des Gorczinskyschen Pyrbeliometers die Gesamtsumme der Sonnenstrahlungsenergie berechnet.

3. Versuchsergebnisse.

1) Lebensdauer der Tuberkelbazillen in den Ausscheidungsmaterialien, die am Fenster des Wohnzimmers aufbewahrt sind. Die Lebensdauer der Tuberkelbazillen in den Ausscheidungsmaterialien, welche am nördlichen Fenster des Wohnzimmers, dessen Glasfenster nach Norden und Westen gehen, aufbewahrt sind, beträgt wie folgt.:

Sputum: Die Tuberkelbazillen im Sputum in der Schale mit Decke leben im Frühling durchschnittlich 13, im Sommer 7, im Herbst 18 und im Winter 23 Tage lang und sterben in 17, 8, 24 bzw. 28 Tagen aus. Im Reagenzglas leben sie im Frühling 27, im Sommer 14 und im Winter 42 Tage lang.

Eiter: In der Schale mit Decke leben sie im Sommer 9 und im Winter 27 Tage lang.

2) Lebensdauer der Tuberkelbazillen in den Ausscheidungsmaterialien, die im Freien im diffusen Licht durch das Glas gelegen sind.

Sputum: Wenn das Sputum in der Schale mit Decke im Freien im diffusen Licht stehen gelassen wird, leben die Tuberkelbazillen im Früling wie im Sommer durchschnittlich 5, im Herbst 7 und im Winter 8 Tage lang. Im Reagenzglas leben sie im Sommer 9 und im Winter 29 Tage lang.

Harn: Im Reagenzglas mit Wattepfropf leben sie im Winter 55 Tage lang.

Kot: Sie leben im Sommer nur einen, im Herbst 3 und im Winter durchschnittlich 7 Tage lang.

3) Lebensdauer der Tuberkelbazillen in den Ausscheidungsmaterialien, die im Frein durch das Glas dem Sonnenlicht ausgesetzt sind.

Sputum: Im direkten Sonnenlicht leben die Tuberkelbazillen in der Schale mit Decke im Frühling wie im Sommer durchschnittlich ein, im Herbst 3 und im Winter 4 Tage lang. Im Reagenzglas mit Wattepfropf leben sie im Winter 21 Tage lang.

Harn: Im Winter leben sie im Reagenzglas durchschnittlich ungefähr 20 Tage lang.

Kot: Wenn wir auch beobachteten, dass sie im Winter 2 Tage lang leben, konnten wir sie doch am 3 Tage und später nicht nachweisen.

Eiter: Ähnlich wie im Sputum leben sie in der Schale mit Decke im Sommer einen und im Winter 3 Tage lang.

Die Gesamtsumme der Sonnenstrahlungsenergie an einem Versuchstage war im Frühling mit durchschnittlich 701.5 Gr. Cal./cm² per Tag

am grössten, dann folgt der Sommer mit 636.0 Gr. Cal./cm² und der Herbst mit 489.6 Gr. Cal./cm². Der Winter ist mit 240 Gr. Cal/cm² am kleinsten.

4) Lebensdauer der Tuberkelbazillen in den Ausscheidungsmateria-

TABLE I.

Zusammenhang zwischen der Lebensdauer der Tuberkelbazillen in Exkreten und dem Somenlicht.

Ort		Ausscheidungs-substanz	Behältnis	Jahreszeit	Lebens-dauer	bis Zum Sterben.	Sonnenstrahlungsenergie
Wohnzimmer am Fenster		Sputum	Schale	Frühling	13 Tage	18 Tage	
				Sommer	7 "	8 "	
				Herbst	18 "	24 "	
				Winter	20 "	25 "	
			Reagenzglas	Winter	42 "	56 "	
				Frühling	27 "	31 "	
				Sommer	14 "	21 "	
		Eiter	Schale	Sommer	9 "	14 "	
				Winter	27 "	42 "	
in Freien in diffusen Licht durch Glas		Sputum	Schale	Frühling	5 Tage	6 "	
				Sommer	5 "	6 "	
				Herbst	7 "	10 "	
				Winter	8 "	10 "	
			Reagenzglas	Sommer	9 "	11 "	
				Winter	29 "	39 "	
		Harn	Reagenzglas	Winter	55 "	67 "	
		Kot	Reagenzglas	Sommer	1 "	2 "	
				Herbst	3 "	4 "	
				Winter	7 "	9 "	
		Eiter	Schale	Winter	10 "	12 "	
		Erde	Reagenzglas	Winter	32 "	34 "	
in direkten Sonnenlicht	durch Glas	Sputum	Schale	Frühling	1 Tage	2 "	701 gr cal/cm²
				Sommer	1 "	2 "	763 "
				Herbst	3 "	3,5 "	489 "
				Winter	4 "	4.4 "	240 "
			Reagenzglas	Winter	21 "	25 "	130 "
		Harn	Reagenzglas	Winter	20 "	25 "	
		Kot	Reagenzglas	Winter	2 "	3 "	
		Eiter	Schale	Sommer	1 "	2 "	
				Winter	3 "	5 "	
	bloss	Sputum	Schale	Frühling	69 min.	93 min.	75 gr cal/cm²st
				Sommer	80 "	107 "	69 "
				Herbst	113 "	143 "	51 "
				Winter	178 "	208 "	38 "
			Glasplatte (Strich)	Frühling	50 "	60 "	67 "
				Sommer	50 "	60 "	62 "
				Herbst	72 "	102 "	42 "
				Winter	2 St.	2st. 30 min.	24 "
			Gaze	Frühling	60 min.	84 min.	69 "
	durch Vitaglas	Sputum	Reagenzglas	Frühling	63 min.	90 min.	66 "
				Winter	135 "	184 "	46 "
		Harn	Reagenzglas	Winter	mehr ald 1 Tag		

lien, die dem direkten Sonnenlicht und ihm durch das Vitaglas ausgesetzt sind. Wenn man 1 ccm Sputum in der Schale ohne Decke dem direkten Sonnenlicht aussetzt, so leben die Tuberkelbazillen im Frühling 50–90, im Sommer auch 50–90, im Herbst 60–210 und im Winter 90–240 Minuten lang und durchschnittlich 69, 80, 113 und 178 Minuten lang. Die Gesamtsumme der Sonnenstrahlungsenergie beträgt dabei per Stunde durchschnittlich 75 Gr. Cal./cm², 69 Gr. al./cm², 51 Gr. Cal/cm² bzw. 38 Gr. Cal/cm², die durchschnittliche Temperatur 13.6°, 24.5°, 14.7°C bzw. 3.1°C, die Feuchtigkeit 67, 87, 77, bzw. 70. Während das Wetter an den Versuchstagen im Frühling und Herbst hell und klar war, war es im Sommer und Winter wegen ziemlich grosser Wolkmenge nicht so klar, daher war der Wert der auf die Erde gelangten Sonnenstrahlungsenergien verhältnismässig niedrig.

Streicht man Sputum dünn aufs Glas aus, so leben die Tuberkelbazillen im Frühling wie im Sommer durchschnittlich 50, im Herbst 72 Minuten und im Winter 2 Stunden lang.

In 1 ccm Sputum auf Gaze, Kunstseide, europäischem Papier und Zellophan leben sie, im Frühling durchschnittlich 61 Minuten lang.

Bei der Anwendung von Vitaglas leben sie, ähnlich wie im direkten Sonnenlicht, im Frühling 63 Minuten und im Winter 135 Minuten lang.

Im Harn leben verhältnismässig viel Tuberkelbazillen, wenn sie auch 7 Stunden lang bestrahlt werden.

4. Zusammenfassung und Betrachtung.

1) Zusammenhang zwischen der Lebensdauer der Tuberkelbazillen in den Ausscheidungsmaterialien und dem Sonnenlicht. Koch[1] hat zuerst mitgeteilt, dass das Sonnenlicht auf die Tuberkelbazillen so sehr bakterizid wirkt, dass sie in einigen Minuten oder 2–3 Stunden aussterben. Von Weinziel,[2] Ogawa[3] und Kiyono[4] u'a. wurden ähnlich Ergebnisse erzielt. Während die Tuberkelbazillen nach Sawitzky[5] im Sonnenlicht zum Aussterben 2.5 Monate brauchen, brauchen sie nach Treskinskaya[6] und Nogami[7] u'a. 4–7 Stunden, nach Donna[8] 6–8 Stunden und nach Mitchel und Crough[9] und Annet[10] 30–48 Stunden. Mit Harn und Kot hat Ogawa[3] experimentell festgestellt, dass sie in einem bzw. 5 Tagen aussterben.

Aus unseren Versuchen ergab sich, dass die Tuberkelbazillen, wenn man ein Sputum gleich nach dem Auswurf dem direkten Sonnenlicht aussetzt, wenn auch je nach dem Tage verschieden, jedoch durchschnittlich ungefähr 2 Stunden lang leben. Diese Resultate sind denen von Koch[1] u.a. nahe, wohl aber leben sie bald 50 Minuten, bald 4.5 Stunden lang, da ihre Lebensdauer von der Sonnenstrahlungsenergie und verschiedenen anderen Bedingungen abhängig ist.

Streicht man Sputum in einer dünnen Schicht aufs Glas aus, so wird die Lebensdauer noch kürzer, sie beträgt nämlich 50 Minuten–2 Stunden, durchschnittlich eine Stunde 13 Minuten.

In dem an Gaze, Kunstseide, europäischem Papier und Zellopan haftenden Sputum ist die Lebensdauer der Bazillen nicht sehr verschieden von dem Fall, wo sie in der Schale aufbewahrt werden. Darum muss man vielleicht Schlafzeuge und Kleidungsstücke zur Desinfektion ungefähr 3 Stunden lang dem Sonnenlichte aussetzen. Downes und Blunt[11] sagten, dass die bakterizide Wirkung des Sonnenlichtes auf den chemischen Strahlen im Sonnenspektrum beruht. Nach Thiele und Wolf[11] ist sie in dem Bereich von 2650–3000 A° und nach Oka[13] nahe von 266 $\mu\mu$ am Stärksten. Jansen[14] u.a. aber sagten, dass die bakterizide Wirkung von dem blauen und grünen Strahlen bis nach dem ultravioletten immer stärker wird. Dagegen behauptete Ducleux,[15] dass sicht bare Strahlen, besonders blaue am stärksten, gelbe und rote etwas schwächer und ultrarote und ultraviolette sehr schwach wirken. Wiesner[16] teilte mit, dass alle Teile des Spektrums eine gleiche bakterizide Wirkung haben und auch ultrarote Strahlen nicht den ultravioletten zurückstehen. Diese Ansicht wurde von Berhard[17] anerkannt.

Aus unseren Versuchen ging hervor, dass, während die Bestrahlung durch das Vitaglas keinen Unterschied von der direkten Bestrahlung des Sonnenlichtes zeigt, bei der durch gewönliches Glas, das Strahlen mit Wellenlänge länger als 3352 A° durchlässt, die Tuberkelbazillen zum Aussterben 1–10 Tage brauchen. Daraus konnten wir folgern, dass ultraviolette Strahlen mit einer Wellenlänge von weniger als 3352 A° am stärksten wirken. Im diffusen Licht im Freien können die Tuberkelbazillen durchschnittlich nur eine Woche lang leben. Während die Tuberkelbazillen, wie in der vorhergehenden Mitteilung geschildert ist, am halbdunklen Orte unter dem Tische oder im dunkelen Wandschrank des Wohnzimmers 3–4 Monate lang leben, können sie am Fenster im Zimmer nur 2–3 Wochen lang leben. Darum kann man sagen, dass das diffuse Licht im Zimmer ebenfalls eine ziemlich strke bakterizide Wirkung hat. Folglich sterben die Tuberkelbazillen in den Ausscheindungsmaterialien im Freien rasch aus und ist die Infektionsgefahr vielmehr im Zimmer gross, besonders im schlecht erleuchteten erheblich.

2) Zusammenhang zwischen der Lebensdauer der Tuberkelbazillen in den Ausscheidungsmaterialien und den Jahreszeiten. Die verschiedenen physikalischen Widerstände der Tuberkelbazillen in den Ausscheidungsmaterialien sind wohl von sehr vielen Autoren untersucht worden, aber der Zusammenhang zwischen ihnen und den Jareszeiten ist doch nur selten geforscht worden. Kita und Nomura[18] teilten mit, dass die

Tuberkelbazillen im Juli und August in einem Tage aussterben, während sie in den anderen Jahreszeiten dazu 7 Tage brauchen. Bergen[9] hat nachgewiesen, dass kultivierte Tuberkelbazillen im Sommer in 30 Minuten, im Früling und Herbst in einer Stunde und im Winter in 2 Stunden aussterben.

An drei Stellen, d.h. am Fenster des Zimmers, im Schatten im Freien und im direkten Sonnenlicht, haben wir die Lebensdauer der Tuberkelbazillen in den Ausscheidungsmaterialien durch die vier Jahreszeiten hindurch ermittelt und verglichen. Dabei stellen wir fest, dass die Bazillen im Sputum im Zimmer im Sommer in einer Woche, im Frühling in 13, im Herbst in 18 und im Winter in 23 Tagen aussterben. Im Schatten im Freien ist ihre Lebensdauer im Sommer und Frühling 5, im Herbst 7 und im Winter 8 Tage. Im direkten Sonnenlicht ist sie im Frühling und Sommer 50–90, im Herbst 60–210 und im Winter 90–240 Minuten lang und durchschnittlich 69, 80, 113 und 178 Minuten lang. Während das Wetter an den Versuchstagen im Frühling hell und klar war, war es im Sommer wegen der ziemlich grosser Wolkmenge nicht so klar, daher war der Wert der auf die Erde wirklich gelangten Sonnenstrahlungsenergien bei weitem niedriger als der Energien, die eigentlich auf die Erde gelangen sollten. Auf alle Fälle ist es sicher, dass je stärker die Sonnenstrahlungsenergie ist, desto kürzer die Lebensdauer der Tuberkelbazillen in Sputum ist. Wenn das Wetter hell und klar ist, ist die Lebensdauer der Tuberkelbazillen im Sputum also im Sommer, wo die Sonnenstrahlungsenergien am stärksten sind, wahrscheinlich am kürzesten, im Frühling etwas länger und im Winter am längsten.

5. Schluss.

1) Setzt man 1 ccm Sputum dem direkten Sonnenlicht aus, so können die Tuberkelbazillen nur äusserst kurze Zeit leben. Ihre Lebensdauer beträgt nämlich im Frühling und Sommer 50–80, im Herbst 60-110 und im Winter 90–240 Minuten lang.

2) Die Tuberkelbazillen im Sputum können im diffusen Licht im Freien im Frühling wie im Sommer nur 5 Tage lang und im Herbst nur eine Woche und im Winter 8 Tage lang leben.

3) Lässt man das Sputum am Fenster des Zimmers stehen, so können die Tuberkelbazillen im Sommer nur 1 Woche, im Frühling 13, im Herbst 18 und im Winter 20 Tage lang leben.

Diese Forschung wurde durch Überlassung von Geldmitteln des Unterrichtsministeriums für wissenschaftliche Forschung ausgeführt, wofür herzlich gedankt sei. Prof. Dr. T. Kumagai und
Prof. Dr. T. Ebina.

Literatur.

1) Koch, R., Schwalbe: Gesammelte Werke von Robert Koch. Lpz., 1912, **1**, 656.
2) Weinziel, J., J. Bacter., 1933, **25**, 447.
3) Ogawa, G., Kekkaku, 1936, **14**, 99; 107: 114.
4) Kiyono, K., Nihon-Biseibutz, Z., 1922, **16**, 7.
5) Sawitzky, W., Zbl. Bakter., 1892, **11**, 153.
6) Treskinskaya, A., Zbl. Bakter., Ref., 1910, **4**, 681.
7) Nogami, Y., Saikin. Z., 1932, 541; 836.
8) Donna, A., Zbl. Bakter., I. orig., 1906, **42**, 642.
9) Mitchell, W. and Crouch, H., J. of path., 1900, **6**, 14.
10) Annett, H. E., J. State Med., 1903, **11**, 462.
11) Downes, A. and Blunt, Th. P., Proc. roy. Soc. Lond., 1877, **26**, 488.
12) Thiele, H. und Wolf, K., Arch. f. Hyg., 1906, **57**, 29.
13) Oka, T., Jikken Iho, 1931, 209, 245.
14) Jansen, Zieglers Beitr., 1907, **41**, 277.
15) Ducleux, E., zit. nach Kekkaku, 1936, **14**, 123.
16) Wiesner, R., Arch. f. Hyg., 1907, **61**, 1.
17) Bernhard, H., Zbl. Bakter., 1.Orig., 1920, **85**, 46.
18) Kita u. Nomura, zit. nach Osaka Igk. Z., 1927, **26**, 1599.
19) Bergen, J., Zbl. Tbk. forschg., 1921, **15**, 343.

Studies on Melanin.

II. The Influence of Benzoquinone on Melanin Production. Preliminary Report.

By

Atsushi Nakajo.
(中條　敦)

(From the Department of Dermatology, Faculty of Medicine, Tohoku University, Sendai. Director: Prof. M. Ito.)

(Received for publication, April 21, 1949)

Artificial production of melanin in yellow-red goldfish was dealt with in this article. Substanced used were *p*-benzoquinone, tyrosine, tryptophane, dioxyphenylalanine and pyrogallic acid (control: saline solution) and daily injected intradermally. The results obtained are as follows:

1. After the fifth injection of *p*-benzoquinone (0,5%), black pigment patche occurs in the scales. The pigmentation takes place earlier in fish with pre-existent lipochrom granules than in pigment-free animal. Pigmentation is seen first in yellow-red macules, and the dopa reaction turns positive at the same time.
2. This pigment-formation was accelerated by simultaneous employment of vitamins A, B_2 and C and glutathione.
3. As in the previous report, temperature and sunlight were found to influence pigment production.
4. The black extract of the newly pigmented scales showed all chemical and physical characteristics of human melanin.
5. *p*-Benzoquinone has special affinity to body fluid of the animal and posesses the action of dopa-oxydase, i.e. the pigment producing facility.
6. Other substances, tyrosine, dopa, pyrrol, adrenaline and copper- and gold-preparations were ineffective in producing melanin at all.

This article was abstracted from the original article in Hifuka-Seibyoka Zasshi (Jap. J. Derma. & Ven.), 1942, **52**. The details will be published in this journal later.

The Tohoku Journal of Experimental Medicine, Vol. 50, Nos. 3 & 4, 1949.

Nachweis von Tuberkelbazillen aus der Bettdecke.

Von

Takesi Abe.
(阿 部 武)

(Aus der Medizinischen Klinik und dem Kosankinbyo Kenkyusyo der Kaiserlichen Tohoku-Universität zu Sendai. Leiter: Prof. Dr. T. Kumagai und und Prof. T. Ebina.)

(Received for publication, April 14, 1945)

1. Einleitung.

In bezug auf den Infektionsweg der Tuberkulose gibt es neben der Luftinfektionstheorie von Koch[1] hauptsächlich drei Theorien: Staubinfektion von Cornet,[2] Schauminfektion von Flügge[3] und Infektion über den Verdauungsapparat von Calmette.[4] Wenn auch die Frage, welche Theorie von grösster Bedeutung ist, noch nicht endgültig gelöst ist, wird seit den Studien von Bruno Lange[5] die Staubinfektionstheorie im allgemeinen als die wichtigste betrachtet. Cornet[2] hat in 47.6% von Staub im Krankenzimmer Tuberkulöser Tuberkelbazillen gefunden und gesagt, dass Staub auf dem Fussboden im Krankenzimmer eine wichtige Infektionsquelle der Tuberkulose ist. Nach Toda[6] ist Staub auf der Matte am gefährlichsten, und nach Lange liegt die Gefahr an allen Orten, nämlich auf dem Fussboden, am Kleidungsstück, auf dem Teppich, auf der Tischdecke usw. Toyama[7] hat Tuberkelbazillen aus 5.8% von Sputum an sehr besuchten Orten in Tokyo, Annaka und Nomura[8] aus 2.3% von Sputum auf den Strassen von Osaka und Saito[9] aus 3.3% von Sputum auf den Strassen von Kumamoto nachgewiesen. Von ihnen wurde das Sputum an den genannten Orten eine wichtige Infektionsquelle der Tuberkulose genannt. Wenn auch Eguti, Yamada und Inoue[10] Versuche mit der wollenen Decke angestellt haben, ist es doch unseres Erachtens noch niemals versucht worden, die Tuberkelbazillen aus dem Bezug an der Bettdecke, der sich an meistens besudelt, nachzuweisen. Im Zweifel, ob Sputum oder schaumiges Sputum an dem Bezug der Bettdecke eine gefährlichere Infektionsquelle der Tuberkulose darstelle als Staub auf dem Fussboden

im Krankenzimmer, nicht zu sprechen von Staub auf der Strasse, haben wir versucht, die Tuberkelbazillen aus dem Bezug der Bettdecke nachzuweisen.

2. Versuchsmethode und -material.

1) *Vorversuche.*

Je 1 ccm von den Emulsionen, in denen 1/100, 1/1,000, 1/10,000, 1/100,000, 1/1,000,000, 1/1,000,0000 mg rein kultivierter Tuberkelbazillen vom Typus humanus enthalten sind, wird auf sterile Gaze (20 cm lang und 15 cm breit) ausgestrichen und nach Behandlung mit drei folgenden Methoden auf 6 Oka-Katakuraschen Nährboden kultiviert, um den Schärfegrad der Methoden zu prüfen und zu vergleichen.

a) 4% Schwefelsäure-Methode: Die Gaze wird in einen Mörser hineingetan und nach Zugabe mit 40 ccm 4% iger Schwefelsäure hinreichend reibend gespült. Die Flüssigkeit wird auf 2 Spitzgläser verteilt und zentrifugiert, und mit dem Bodensatz wird die Kultur durchgeführt.

b) Katakura-Oka[11]sche Zink-Sulfat-Methode: Die Gaze wird mit 40 ccm 2% NaOH hinreichend gespült. Nachdem die Flüssigkeit in 2 Spitzgläser verteilt worden ist, wird sie nach Zusatz mit 2–3 ccm von 2.5% $ZnSO_4$ zentrifugiert. Der Bodensatz wird dann nach Behandlung mit 10 ccm von 4%H_2SO_4 kultiviert.

c) Katakura-Oka[4] sche Schwefelsäure-Pepsin-Methode: Die Gaze wird mit 40 ccm des Gemisches von 0,1% Saponin und 0,25% $Cacl_2$ gespült. Nachdem die Flüssigkeit in 2 Spitzgläser gegossen worden ist, bekommt sie je 1 ccm Blut von gesunden Kaninchen und wird 3 Stunden lang stehen gelassen. Nach Zentrifugieren wird der Niederschlag mit sterilisiertem destilliertem Wasser gespült. Der Bodensatz wird nach Zugabe mit 3 Tropfen von 4% Schwefelsäure, in der 5% Pepsin enthalten sind, wieder zentrifugiert, und mit dem Sedimente wird die Kultur ausgeführt.

2) *Versuche mit dem Bezug am Kopfende der Bettdecke.*

In der Mitte des Bezuges am Kopfende der Bettdecke von 119 Lungentuberkulösen in unserer Klinik wurde Gaze von der oben angegebenen Grösse befestigt und eine Woche lang stehen gelassen. Dann wurde sie in einen aseptischen Mörser hineingetan und mit der Zink-Sulfat-Methode verfahren. Die Flüssigkeit wurde auf 6 Oka-Katakura schen Nährboden kultiviert, und durch die 2 Monate lange Beobachtung wurden die Ergebnisse beurteilt.

Tabelle I.

Kulturresultate aus der Gaze mit ausgestrichener Bazillenemulsion.

Methode \ Konzentration		1/100 mg	1/1,000 "	1/10,000 "	1/100,000 "	1/1,000,000 "	1/10,000,000 "
Gaze	H_2SO_4	3/6	1/6	3/6	0	0	0
	$ZnSO_4$	100/6	20/6	27/6	4/6	3/6	0
	H_2SO_4+ Pepsin	18/6	18/6	10/6	2/6	1/6	0

{Zähler Zahl der Kolonien
{Nenner Zahl der Nährboden

Tabelle II.

Zusammenhang zwischen den Fällen mit positiven Ausfall und Kaverne, Sputummenge oder Bazillenausscheidung.

Bazillen in Sputum		gesamte Fällen der Versuche						positive Fällen					
		Kaverne		Sputummenge			Summe	Kaverne		Sputummenge			Summe
		+	−	###	##	+		+	−	###	##	+	
In Ausstriche	###	23	7	14	16	0	30	8	2	6	4	0	10 33.3%
	##	12	2	2	5	7	14	3	0	1	1	1	3 21.4%
	+	12	14	0	8	18	26	3	2	0	1	4	5 20.8%
	Summe	47	23	16	29	25	70	14 29.7%	4 17.3%	7 43.7%	6 20.6%	5 20.0%	18 25.1%
Kultur	+	1	31	0	3	29	32						0
	−	5	12	0	2	5	17						0
Gesamtsumme							119						18

in Ausstriche {### mehr als 10 Bazillen in einem Gesichtsfeld
{## mehr als 2 " "
{+ weniger als 2 " "

Sputummenge {### mehr als 30 spute tägich
{## " 10 "
{+ wenig als 10 "

3. Versuchsresultate.

1) Vorversuchsresultate. Aus den Resultaten der Vorversuche, ging hervor, dass sowohl die Zink-Sulfat-Methode als auch die Schwefelsäure-Pepsin-Methode sehr scharf sind und man dadurch Bazillen bis 1/1,000,000 mg nachweisen kann. Da die erstere Methode einfacher ist, ist sie für den Nachweis von geringer Menge Tuberkelbazillen geeignet.

2) Ergebnisse der Versuche an dem Bezug am Kopfende der Bettdecke.

a) Prozentsatz des positiven Nachweises von Tuberkelbazillen. Von insgesamt 119 Fällen zeigen 18 positiven Ausfall. Sie gehören alle zu denjenigen an, bei denen Bazillen aus Sputum mikroskopisch nachweissbar sind, und machen 25,7% von 70 offenen Versuchspersonen aus. 10 (33,3%) davon gehören zu 30 Fällen mit grosser Bazillenausscheidung (durchschnittlich mehr als 10 Bazillen in einem Gesichtsfeld), 3 (21,4%) zu 14 Fällen von mittelgradiger Bazillenausscheidung (durchschnittlich 2–10 Bazillen in einem Gesichtsfeld) und 5 (20,8%) zu 26 Fällen von kleiner Bazillenausscheidung (durchschnittlich weniger als 2 Bazillen in einem Gesichtsfeld).

b) Kolonienzahl der positiven Kultur. Auf 6 Nährboden sind 4 und 3 Kolonien bei je einem Falle aufgetreten, und 2 Kolonien bei 3 Fällen. Die Fälle, bei denen nur eine Kolonie erschienen ist, sind am meisten, nämlich 13. Die Fälle, denen mehr als 2 Kolonien aufgetreten sind, gehören zu denjenigen an, bei denen viel Tuberkelbazillen ausgeschieden werden.

c) Zusammenhang zwischen den Fällen mit positivem Ausfall und der Kaverne.

Bei 14 (29,7%) unter 47 Fällen mit Kaverne, bei denen der Bazillenbefund auf Ausstriche positiv ist, fällt die Kultur positiv aus, und bei 4 (17,3%) unter 23 ohne Kaverne, bei denen ebenfalls Bazillen auf derselben Weise nachgewiesen werden, zeigt die Kultur positiven Ausfall. Zwei von den 4 Fällen ohne Kaverne sind an Kehlkopftuberkulose Leidende.

d) Zusammenhang zwischen den Fällen mit positivem Ausfall und dem Katarrh, insbesondere der Sputummenge. Bei 7 (43,7%) von 16 Fällen, bei denen mehr als 30 Sputa täglich ausgeworfen werden, bei 6 (20,6%) von 29 mit 11–29 Sputa und bei 5 (20%) von 25 mit veniger als 10 Sputa werden die Tuberkelbazillen kulturell nachgewiesen. Bei den Fällen mit Katarrh und grosser Sputumemnge werden Tuberkelbazillen mehr nachgewiesen.

4. Zusammenfassung und Betrachtung.

Staub auf dem Fussboden und Sputum auf der Strasse wurden von verhältnismässig vielen Autoren als wichtige Quellen der Tuberkuloseinfektion durch die Luftwege betrachtet und der Nachweis der Tuberkelbazillen daraus wurde oft versucht, allein es wurde nur von wenigen versucht, sie aus dem Bettzeuge nachzuweisen. Ausser der Angabe von Eguti, Yamada und Inoue,[10] welche Meerschweinchen Staub auf der wollenen Decke impften und dadurch bei 5 von 6 Versuchstieren tuberkulöse Veränderungen hervorriefen, liegt fast keine von der Art in der Literatur

vor. Aus dem Bezug am Kopfende der Bettdecke, der sich am erheblichsten besudelt, scheint noch niemand Tuberkelbazillen nachgewiesen zu haben. Daher haben wir mit dem Spülwasser des Bezuges die Kultur der Tuberkelbazillen durchgeführt, um den Grad der Gefahr der Infektion durch den Bezug klar zu machen. Als Vorversuche haben wir auf sterilisierter Gaze Tuberkelbazillen ausgestrichen und nach Behandlung mit der Zink-Sulfat-Methode und mit der Schwefelsäure-Pepsin-Methode kultiviert. Da wir aus den Ergebnissen der Vorversuche festgestellt haben, dass man durch die Methoden sehr geringe Menge Tuberkelbazillen von 1/1,000,000 mg nachweisen kann, so haben wir eine sterile Gaze von der angegebenen Grösse in der Mitte des Bezuges am Kopfende der Bettdecke befestigt und nach einer Woche mit der Zink-Sulfat-Methode, deren Verfahren einfacher ist, versucht, Tuberkelbazillen daraus nachzuweisen. Aus den Resultaten ist es festgestellt, dass der Bezug am Kofende der Bettdecke von offenen tuberkulösen Personen beträchtlich von den Tuberkelbazillen besudelt wird und besonders bei den Fällen mit Kavernen erheblich. Da die Tuberkelbazillen im Sputum nach den Resultaten unserer Versuche am Fenster 7–23 Tage, unter dem Tische 77–89 Tage lang leben, ist es höchst wahrscheinlich, dass schaumiges Sputum oder Speichel eine sehr gefährliche Infektionsquelle werden, indem sie trocken werden und sich vom Kleidungsstücke nach allen Richtungen hin zerstreuen.

5. Schluss.

1) Wenn man eine Bazillenemulsion auf Gaze ausstreicht, spült und nach Behandlung mit der Zink-Sulfat-Methode oder mit der Schwefelsäure-Pepsin-Methode kultiviert, so kann man die Tuberkelbazillen von Typus humanus in 1/1,000,000 mg Konzentration nachweisen. Dabei werden 1–4 Kolonien auf 6 Nährboden herausgezüchtet.

2) Wenn auch die Besudelung in der Mitte des Bezuges am Kopfende der Bettdecke bei offenen Tuberkulösen am erheblichsten ist, fällt die Kultur aus dem Bezug bei 33,3% von den Fällen mit grosser Ausscheidungsmenge von Bazillen, bei 21,4% von den mit mittelgradiger Ausscheidungsmenge von Bazillen und bei 20,8% von den mit geringer Ausscheidungsmenge von Bazillen, also durchschnittlich bei 25% positiv aus. Besonders bei den Kavernen oder den mit Kehlkopftuberkulose komplizierten, bei denen der Katarrh erheblich ist, ist der Prozentsatz der positiven Ausfälle hoch.

3) Aus dem Bezug von den Fällen, bei denen keine Tuberkelbazillen auf Ausstriche gefunden werden, fällt der Nachweis von Tuberkelbazillen negativ.

4) Die Besudelung des Bezuges bei den Fällen, bei denen der Billen-

befund auf Ausstriche positiv ist, entspricht der Bazillenmenge von 1/1000-000 mg. in einer Flächeninhalt von 300 cm².

Diese Forschung wurde duch Überlassung von Geldmitteln des Unterrichtsministeriums für wissenschaftliche Forschung ausgeführt, wofür herzlich gedankt sei. Prof. Dr. T. Kumagai und
Prof. Dr. T. Ebina.

Literatur.

1) Koch, R., Schwalbe: Vesammelte Werke von Robert Koch. Lpz., 1912, **1**, 443.
2) Cornet, G., Z. Hyg., 1889, **5**, 191.
3) Flügge, C., Z. Hyg., 1899, **30**, 107.
4) Calmette, A., Zit. nach Nihon Ij. Sp., 1923, **111**, 2.
5) Lange, B., Zbl. Bakter., Abt. I. Orig., 1924, **93**, 66.
6) Toda, S., Nihon Ij. Sp., 1923, **111**, 2.
7) Toyama, S., Kenbikyo, 1902, **53**, 1.
8) Annaka und Nomura., zit. nach Kumamoto Isen. Koyu. Z., 1915, **19**, 64.
9) Saito, S., Kumamoto Isen. Koyu. Z. 1915, **19**, 64.
10) Eguchi, I., Yamada, S., und Inoue, Y., Gun-i. Z., 1936, **278**, 929.
11) Katakura, T. Oka, S., Isikawa, G. und Okamura, K., Tohoku Ig. Z., 1939, **24**, 71

The Tohoku Journal of Experimental Medicine, Vol. 50, Nos. 3 & 4, 1949.

Ein Fall von Adenocarcinoma cysticum des Magens.

Von

Kenji Honda u. Tōru Watanabe.
(本多 憲兒)　(渡邊 達)

(Aus der chirurgischen Klinik der Universität, Sendai.
Direktor: Prof. Dr. M. Muto.)

(Received for publication, September 29, 1946)

Im folgenden soll ganz kurz über einen Fall von Adenocarcinoma cysticum des Magens berichtet werden, bei dem wir uns für sein seltenes histologisches Pild interessierten.

Ein 44 jähriger Mann, der seit 2 Jahren Magenbeschwerden hatte, und dessen Erkrankung in der med. Klinik von Prof. T. Kurokawa klinisch als Magenkrebs mit Pylorusstenose und röntgenologisch als Schüsselkrebs festgestellt worden war, kam zu uns zur Operation. Glatte Heilung durch Magenresektion.

Im Pylorusteil des resezierten Magens war, vorwiegend in seiner vorderen Wand, ein hühnereigrosser Tumor vorhanden, der mit einigen subserösen, bis bohnengrossen, dunkelbläulich durchschimmernden Cystchen in der Mitte versehen war. In der Innenfläche des Magens, und zwar entsprechend dem Sitz des Tumors fand sich, wie Abb. 1 zeigt, ein hühnereigrosses schüsselförmiges Geschwür. Auf einigen Schnitten des Tumorgewebes (siehe Abb. 2) sah man zahlreiche, nadelspitz- bis bohnengrosse Cystchen, die in allen Schichten dicht beieinander lagen (siehe Abb. 3) und bald schwärzlich bräunliche, bald blau grünliche, geronnene Massen enthielten.

Mikroskopisch erschien die Oberfläche des Geschwürbodens mit einem dünnen Granulationsgewebe, hier und da über diesem mit Nekrosemasse bedeckt. Unter dieser Granulationsschicht lag Tunica muscularis. In allen übrigen Schichten des Geschwürbodens und auch in seiner submukösen Randschicht sah man zahllose, verschieden grosse, unregelmässig verschieden geformte, drüsenähnlich oder zystisch erweiterte Gebilde. Die Muskelbündel waren dadurch zerbrochen, aber doch noch erhalten. Diese Drüsen und Cystchen waren meist unilokular, zuweilen auch mehrkammerig. Ihre Epithelzellen waren entsprechend

ihrer Lumengrösse endothelähnlich platt, kubisch, kurz- oder hochzylindrisch. Die grösseren waren nämlich mit einschichtigem abgeplattetem Epithel ausgekleidet und enthielten meist homogene geronnene Massen mit Leukocyten oder Exsudat mit Schleimbeimengung. Die kleineren Drüsen waren meist mit einschichtigen kubischen Epithelzellen ausgekleidet, die aber an vielen Stellen unregelmässig wucherten. Nicht selten sahen wir unter ihnen papilläre Wucherungen der Epithelschicht nach innen oder Durchwucherung der Epithelzellen durch die Basalmembran nach aussen in das umgebende Stromagewebe. Ausserdem bemerkten wir hier und da solide Zellwucherungen der Epithelzellen ohne Lumenbildung, dies besonders häufig in der Schleimhaut des Geschwürrandes. Diese Wucherzellen waren meist polyedrisch oder rundlich und besass meist chromatinreiche Kerne, verhältnismässig häufig mit Kernteilungsfiguren.

Es handelte sich also bei diesem Fall um einen Magentumor mit ausgedehnter Geschwürbildung. Der Tumor bestanden zum grössten Teil aus adenomatösem, adenocystomatösem und cystomatösem Gewebe, das aber in einem Teil beginnende maligne Entartung zeigte. Schliesslich fand man das carcinomatöse Bild vorwiegend am Geschwürrand. So musste der Tumor als Adenocarcinoma micro- et macrocysticum des Magens und zwar als eine Abart von Carcinoma microcysticum (Ribbert) oder von Cystocarcinoma papilliferum (Bormann, R.: Henke Lubarsch'sches Handbuch, Berlin, 1926, Bd. 4, T. 1. S.873–875) angesprochen werden, obwohl diese Form des Magencarcinoms in der Literatur nicht gefunden werden konnte.

Bezüglich der Pathogenese unseres Tumors nehmen wir an, dass das sog. Ulcusadenom im bereits vorhandenen Geschwürboden entstand, das sich einerseits cystisch erweiterte, anderseits malignös entartete, und dass eine primäre Krebsentstehung mit Sicherheit ausgeschlossen werden kann, da kein Krebsgewebe sondern nur Granulationsgewebe an der Oberfläche des Geschwürbodens bemerkt wurde (von Herrn Prof. O. Kimura, Direktor des hiesigen Pathologischen Institut bestätigt).

Abb. 1.

Abb. 2.

Abb. 3.

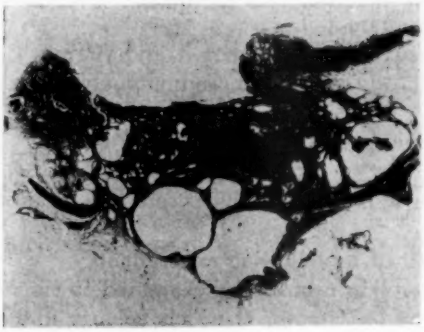


Abb. 4.

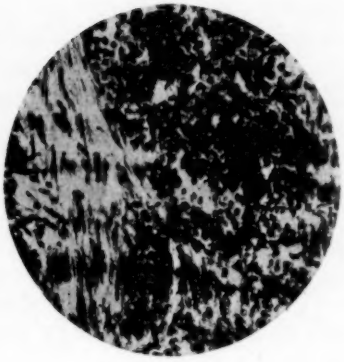


Abb. 1. Innenfläche des resezierten Magens. Der Magen in seiner hinteren Wand längs aufgeschnitten. P: Pylorus, G: Geschwür.

Abb. 2. Schnittfläche des Tumorgewebes. Im Geschwürboden (G) ist die Schleimhaut völlig verschwunden und die Muskelschicht blossgelegt. Hier ist die Magenwand durch Tumorgewebe mit zahlreichen Cystchen durchgesetzt und 2 bis 3-fach dicker als die sonstige Wand.

Abb. 3. Lupenvergrösserung eines histologischen Präparates. Die verdickte Magenwand ist mit zahlreichen Cystchen durchsetzt und sieht wabig aus.

Abb. 4. Drüsen mit einschichtigen kubischen Epithelien in der Tunica muscularis. Man sieht beginnende Durchwucherung der Epithelzellen einer Drüse durch die Basalmembran nach aussen. Mittelstarke Vergrösserung.

Studies on Melanin.

III. Ehe influences of Bergamotoil Applied Together with Vitamin C on Melanin Production. Preliminary Report.

By

Atsushi Nakajo.

(中條　敦)

(From the Department of Dermatology, Faculty of Medicine, Tohoku University, Sendai. Director: Prof. M. Ito.)

(Received for publication, April 21, 1949)

In 1937 Urbach and Kral found that bergamotoil, when applied with vitamin C, remarkably protects pigment production after ultraviolet irradiation. These authers attributed this to the antiallergic activity of vitamin C against sensitisation to bergamotoil.

The writer obtained similar results with bergamotoil and vitamin C. However, unexplainable were the following facts by mere immunobiological theory of Urbach and Kral.

1. In case other photodynamic substances were used, vitamin C failed to display any protection against pigmentation.

2. Vitamin B_2 and adrenalin also exerts similar protection against bergamotoil.

These contradictions are explained by following experiments.

1. The penetration of bergamotoil in rabbit's skin examined by Sudan III staining, was markedly reduced by vitamin C which belongs to the negative group.

2. The oxygen-space, examined by Unna's method, was considerabl increased both in epidermis and corium after the application of bergamotoil alone. Applied with vitamin C, however, bergamotoil-spreading failed to produce such increase of oxygen-space, while coal tar was not influenced by vitamin C.

Such difference between coal tar and bergamotoil is ascribed to being oxidable of bergamotoil contrary to coal tar.

From the above mentioned facts, it is justified to assume that vitamin C accomplishes its inhibitory action against pigment production by decreasing penetrability of the skin, and bergamotoil itself has reducing force so that it inhibits pigment production.

This article was abstracted from the original article in Hifuka-Seibyoka Zasshi (Jap. J. Derma. & Ven.), 1942, **52**. The details will be published in a later issue of this journal.

The Tohoku Journal of Experimental Medicine, Vol. 50, Nos. 3 & 4, 1949.

Biochemical Studies on Carbohydrates.

C. Group Specific Substances in Gastric Mucosa.

First Communication.

By

Hideo Sibuya.

(澁谷 英郎)

(From the Medico-Chemical Institute, Tohoku Imperial University, Sendai. Director: Prof. H. Masamune.)

(Received for publication, October 10, 1946)

In 1937 Meyer et al[1] separated an A group specific "neutral" polysaccharide from "mucin" of pig gastric mucosa. Whether the mucosa here was the one that had been removed from the covering mucus or not is not mentioned in the report and therefore it is uncertain if the polysaccharide they obtained came fom the mucus or from the proper mucosa, and furthermore one can not tell from their experiments if the substance originated in a glycidamin (Masamune[2]) or is present in the tissue in a free state, because they adapted alkaline treatment for preparation. The specimens contained hexuronic acid and their discordant quantitative composition is supposed to reveal themselves quite impure. Similar consideration will be given to the substance separated early by Brahn, Schiff and Weinmann[3] from pepsin.

We could isolate two A group substances, one a carbohydrate and the other a glycidamin from pig gastric mucosa from which mucus had been scraped off as completely as possible. Throughout the whole course of preparation drastic operations were avoided. Namely, the substances extracted with water were divided into acetic acid-precipitable and soluble parts, of which the former gave, after purification with sodium bicarbonate and toluene, the glycidamin (Substauce G) free from phosporus and the latter, on electrodialysis followed by fractioning with glacial acetic acid, the carbohydrate which was insoluble in glacial acetic acid—Substance C. C contained no phosphorus nor hexuronic acid and found to be composed of equimolecular glucosamine and galactose (regarding identification, see the forthcoming paper by Masamune, Sibuya and Tiba[4]), resembling the A substance in the mucus mucin of the same stomach[5], but, in divergence therefrom, had no sulfate rest and exhibited coloration with Ehrlich's *p*-dimethylaminobenzald-

ehyde reagent only after treatment with hot alkali (Osaki and Turumi procedure[6]). Since the present carbohydrate was equally or even more inhibitory to isoagglutination of A erythrocytes than that of the mucus mucin, the mentioned rest and Blix direct reaction[7]-giving structure which the latter possesses are assumed dispensable for this serological activity. In the qualitative as well as quantitative (molecular ratios of the components) analytical respects noted above the polyose moiety in substance G accorded with C, suggesting that G is made up of an amino acid-grouping and C. From what Sinokawa[5] claimed, the carbohydrate separable from the mucus-mucin is the only one in the mucus that is insoluble in glacial acetic acid. Therefore not only substances C but also G are deemed to pertain to the proper mucosa tissue.

Primarily the present work was undertaken to isolate in chemically pure form the poison which had been noticed by Hirooka[8] in the acetic acid-precipitable fraction (" mucin ") of the mucosa (adhered with mucus) extract. The toxicity of the " mucin " is said to be neither destroyed by digestive secretions (gastric, pancreatic and intestinal juices), though not perfectly resistent, nor reduced by dialysis. This finding attracted our attention, because, in the first place, the preparations had phosphorus, as the auther himself remarked, while most tissue poisons are characterised by promoting blood coagulation which is effected by kephalin, a phosphorus ocmpound, or so-called tissue coagulant, an addition product of kephalin and protein. In the second place, gastrin in the stomach mucosa is looked at by some investigators as the very toxic histamine and such polar crystalloids as this are not readily removed by ordinary dialysis, when adsorbed to a protein.

It is also reported[9] that stomach mucosa involves a hemolytic substance. But this alleged unsaturated fatty acid dissolves in methanol as well as in ether, which Hirooka used for washing the acid-precipitates.

To start with, we liberated, after attempts, the mucin from the phosphorus-containing contaminants and examined if it still remains toxic. 5–10 mgm. of the final product (Substance G) killed a mouse of 10 gm. body weight, when intraperitoneally applied, but at a certain stage of purification was sometimes found that the toxicity of the substance was lowered. So we searched for another poison in the mother liquid after precipitating the tissue extract with acetic acid (see the preparation procedure in the exp. part). It was caused thus to gain the carbohydarate Substance C. Though devoid not only of phosphorus but also of histamine (Hanke and Koessler), the preparations, which were water insoluble, generally exhibited toxicity of a minimun lethal dose as small as 1 to 2 mgm., but some obtained in summer-time were much less poisonous. It

appeared probable that its poisonousness consists in high molecular association and in hot season some manipulation or slight putrifaction during preparation splitted up the association, because the closely related group-specific substance from the mucus-mucin is water soluble and simultaneously non-toxic. Viewing thus, we treated a fully toxic specimen with cold 1 n. NaOH solution to change it soluble in water. The recovered substance showed the quantitative analytical figures and serologinal activity same as the orignal, whereas it developed no intoxication symptom even in a dose of 10 mgm. In this way, however, one can not yet derive a decisive conclusion, because a possible real poison admixed to the carbohydrate may have been decomposed by alkali or diffused out during dialysis (s. below). A further study is in progress.

EXPERIMENTAL.

Phosphorus-free Mucin from Hog Stomach Mucosa. (Preparation Method and Properties.)

The mucosa and mucus of hog stomachs were teared off together from the muscular layer and they were separated from each other most possibly in a mechanical way. After washing under running water, the mucosa was minced and agitated with 5 volumes of water for 24 hours. Putrifaction was prevented by addition of alcoholic thymol solution (20%). To the viscid yollowish liquid, separated by certrifugalization and percolation through a gauze, was added acetic acid (30%) in drops to maximum precipitation (pH, round 4). Extraction was repeated (more 3 times) as far as acetic acid-precipitable substances came over and the flocculent precipitates were united (the mother liquids were used for isolation of the group-specific carbohydrate (Substance C)), washed with water (2–3 times) at a similar pH (acetic acid was used) as above[10] and again taken up in water of an equal volume as that used for each extraction. The suspension was then acidified to pH 2.0 (or less) by careful addition of 1 n. HCl solution, whereupon nearly half of the flakes went into solution. The remainder undissolved was centrifuged, washed with a dilute HCl solution at pH 2.0[11] and after redissolving within pH 7–8 by the aid of saturated baryta water (the insoluble part was centrifuged off), precipitated at pH 2.0 by means, of HCl. At the end the precipitates were washed with alcohol and ether and dried. It still contained much phosphorus.

Purification was carried out on the principle of Sinokawa[5] as follows:—The crude product was kneaded with toluene and stood for 1 or 2 days. Toluene was poured out and the substance shaken on a machine with 1% $NaHCO_3$ solution (300 cc. for 1 gm. substance) and

a new portion of toluene overnight. On centrifugation the mucin gathered together in the upper toluene layer. The lower water layer was siphoned out and the gel was further treated many times[12] with the bicarbonate solution and toluene and shrunk in water by acidifying to pH 2.0, when all phosphorus had been eliminated. After washing with water, alcohol and ether in sequence, it was dried in vacuo.

With dimethylaminobenzaldehyde the substance obtained gave a red dye, only when heated with a sodium carbonate solution in advance(Osaki-Turumi procedure[6]). Hydrolysable sulfur was absent. Intensive Molisch reaction (bright violet) occurred. Quantitative analysis of the preparations gave figures: N (micro Kjeldahl), 11.5–12.2%; hexosamine (10.0–11.0%); galactose (the indole, the orcinol and the diphenylamine I method), 10.5–11.3%; acetyl (Suzuki's modification of Friedrich, Rapoport and Sternberg method), 2.40–2.60%; ash (Pregl), 2.0–2.4%. 5–10 mgm. were the smallest quantity killing a mouse of 10 gm. body weight, when injected intraperitoneally (M.L.D.). Specific inhibition of A erythrocyte isoagglutination was exhibited up to $1:10^6$–10^7 dilution by the way of test higherto employed in this laboratory (serum titres: A, $\times 160$, B, $\times 80$).

The Group Specific Carbohydrate (Substance C).

The supernatant liquids after centrifugation of the acidified mucosa extracts (s. above) were combined and as much baryta (a saturated solution) was added as to bring the pH to 9.0. The flocculent precipitates involving the large part of phosphorus were separated off on a folded filter and the filtrate was acidulated with acetic acid (30%), dialyzed (in a urine bladder) against running tap water for 3 days (pH was raised to 6–7) and after the appearing deposit was centrifuged off, distilled under reduced pressure. The syrupy residue was dehydrated with absolute alcohol, washed with absolute alcohol and ether and dried in vacuo. About 1 gm. of the substance was yielded from 300 gm. of the fresh mucosa (average weitght of mucosa obtainable from one stomach). Several trials were made to get rid of the remaining phosphorus. Among others electrodialysis (100 volts, collodion membrane) was most useful.

1% solution was electrodialyzed. After 3 days, a part of the substance which was thrown down meantime and that in solution in the middle compartment were dried separately, by washing the former directly with absolute alcohol and ether followed by placing in vacuo, and by treating the latter likely after distilling (alcohol was added from time to time) to a syrup in vacuo. Since the amount of phosphorus was found markedly reduced, the fractions were again subjected to the operation. Here the deposit by the first dialysis was suspended at neutral reaction by the use

of Na_2CO_3 (5% solution). A trace of phosphorus was still detected after the same duration of dialysis as above, but it disappeared finally in the third run.

By electrodialysis phosphorus was taken off but biuret-giving substances were not. Therefore we further resorted to fractionation with glacial acetic acid. Each of the fractions above, water-soluble and -insoluble, was kneaded into a thick paste with a little water and glacial acetic acid (99-100%) was added, at first drop by drop each being subsequently mixed with the paste to obtain a homogeneous solution, and afterwards in larger portions until no more precipitation occurred. The precipitates were washed with alcohol exhaustively and dried by substituting alcohol for ether and placing in vacuo. The treatment was carried out over and over again and stopped when glacial acetic acid-soluble (ether-insoluble) impurities were no more present.

The preparations from the fractions soluble in water and those from the insoluble showed no difference in all the respects following. They were non-colored and insoluble in plain water and analyzed: N, 4.2-4.5%; glucosamine, 40.0–41.8%; galactose, 39.7–42.0%; acetyl, 10.0-10.67%; ash, 1.10–1.51%. They contained no hydrolysable sulfur. Biuret and ninhydrin tests resulted in negative, but arginine test (Sakaguchi) in positive. Osaki-Turumi test: indirect reaction, positive; the direct, negative. M.L.D. was 1–2 mgm. A group specific activity was demonstrated at $1:10^7$ dilution[13].

Treatment with Alkali of the Carbohydrate.

To 100 mgm. of Substance C was dropped 1 n. NaOH solution under cooling in water. The total volume of the NaOH solution used was 5 cc. On standing in air (19–23°C) with occasional shaking, it gradually went into solution. After 27 hours, a small insoluble part was centrifuged off. The supernatant fluid was diluted to 30 cc. with distilled water and acidulated with glacial acetic acid (to pH 4.0, no heat evolution), whereupon slight turbidity appeared. Centrifuged. The clear liquid was dialyzed (an intestine bag), after addition of thymol (alcoholic solution), against many changes of distilled water. The dialyzed liquid was condensed at a low temperature to a syrup, absolute alcohol and sodium acetate added, and the precipitates washed with absolute alcohol and ether and dried. 35 mgm. of white powder were obtained. It was soluble in water and analyzed: N, 3.9%; glucosamine, 42.2%; galactose, 41.9%. The colour reaction with *p*-dimethylaminobenzaldehyde after preliminary heating with alkali (acetylglucosamine test) and arginine reaction were given. Ninhydrin test, negative. No toxic symptom was

observed at all when 10 mgm. of it for 10 gm. body weight were applied to a mouse in a way as above, but the isoagglutination-inhibitory power was seen completely retained.

The costs of this work were defrayed in part by a grant from the Grant Committee for Scientific Researches of the Education Department, which is gratefully acknowledged.

Bibliography and Notes.

1) Meyer, Smyth and Palmer, J. Biol. Chem., 1937, **119**, 73.
2) Masamune, Tohoku J. Exp. Med., this volume, p. 107.
3) Brahn, Schiff and Weinmann, Klin. Wochens., 1932, **11**, 1592.
4) Masamune, Sibuya and Tiba, Tohoku J. Exp. Med., in press.
5) Kobayasi, J. Bioch., 1941, **33**, 369. Sinokawa, Tohoku J. Exp. Med., this volume, p. 167.
6) Osaki and Turumi, Tohoku, J. Exp. Med., 1947, **49**, 11.
7) Blix, Z. f. physiol. Chem., 1936, **240**, 43.
8) Hirooka, Tohoku J. Exp. Med., 1942, **42**, 309.
9) Faust and Tallqvist, Arch. exp. Pathol. u. Pharm., 1907, **57**, 367.
10) In one run, where 416 gm. of the fresh mucosa were used, the crude mucin at this stage weighed 2.2 gm. after washing with alcohol and ether and drying.
11) The substance was reduced to 1.35 gm., when preparation was started with another 416 gm. portion of the mucosa batch noted in 10).
12) It is recommendable to shrink the mucin half way with dilute HCl, if its too much swelling makes difficult further treatment.
13) The substance was swollen in advance in the physiological saline solution by means of Na_2CO_3.

The Tohoku Journal of Experimental Medicine, Vol. 50, Nos. 3 & 4, 1949.

Biochemical Studies on Carbohydrates.

CI. Group Specific Substances in Gastric Mucosa.

Second Communication.

By

Hajime Masamune, Hideo Sibuya and Hirosi Tiba.

(正宗 一) (澁谷 英郎) (千葉 博)

(From the Medico-Chemical Institute, Tohoku Imperial University, Sendai.)

(Received for publication, October 10, 1946)

The group specific carbohydrate of pig gastric mucosa, which one of us has informed of in the foregoing article of this series[1], was prepared in a simpler method and after fractionation of the acid hydrolysate, the sugar components were identified with glucosamine and galactose as the pentabenzoate and methylphenylhydrazone respectively.

EXPERIMENTAL.

Procedure of Preparing the Group Specific Carbohydrate.

The acid-unprecipitable fraction of the mucosa extract was obtained according to Sibuya[1]. To 0.5 gm. of it were added several drops of glycol at first and then small portions of glacial acetic acid (99%), kneading thoroughly each time. The viscid solution thus obtained was precipitated by increasing glacial acetic acid to about 30 cc. and centrifuged. The centrifugate became free from phosphorus on treating similarly more two or three times. (When the supernatant liquid remained turbid which occurred in a repeated run, addition of a little anhydrous sodium acetate could clarify it). The substance was then washed with alcohol over and over till all acetic acid disappeared (in this case also the use of sodium acetate was needed) and finally dried in a vacuum desiccator (H_2SO_4). Usually the crop at this stage was about 0.2 gm.

The above product, which still gave biuret reaction, though weak, was extracted with 20 cc. portions[2] of 10% trichloracetic acid exhaustively. To clarify the extracts, prolonged centrifugation and frequent change of the centrifuge tube were required. 3 volumes of acetone and a little anhydrous sodum acetate were added and the precipitates were washed

with alcohol repeatedly to remove trichloracetic acid and dried as above. Yield in averages, 80 mgm.

The final substance was difficultly soluble in plain water and biuret-negative. One of the preparations gave quantitative figures: N, 4.57%; glucosamine, 42.9%; acetyl, 10.39%; galactose, 42.5%; ash, 3.19%. Others analyzed similarly. Isoagglutination of A erythrocytes was inhibited at a dilution of $1:10^7$.

Identificatrin of Glucosamine and Galactose in the Carbohydrate.

188 mgm. of the polysaccharide prepared as above were submitted to acid hydrolysis (10 cc. of 1 n. H_2SO_4 solution were used; duration of heating, 5 hours) and the following operations which Tiba adapted for the same purpose in the 97th investigation of this series[3].

Glucosaminepentabenzoate. The initial crystalline product (m. p., 207°C) amounting to 57 mgm. was recrystallized from methanol three times and 26 mgm. of a preparation with constant melting point (215°C) yielded. N(micro Kjeldahl), 2.08% (calc., 2.00%). $[\alpha]_D^{13}$ (in pyridine) $= \frac{0.29 \times 3.0125}{0.01944 \times 0.9835 \times 1} = +45.7°$.

Galactose methylphenylhydrazone. The crude product (38 mgm.) (m. p., 180°C) was recrystallized (three times) to a constant melting point (189°C). Yield, 19 mgm. Mixed melting point test showed no depression. N (micro Dumas), 9.93% (calc., 9.85%).

The costs of this work were covered by a grant from the Grant Committee for Scientific Researches of the Education Department.

Bibliography and Notes.

1) Sibuya, Tohoku J. Exp. Med., this volume, p. 355.
2) 3–5 portions.
3) Tiba, Tohoku J. Exp. Med., this volume, p. 281.

The Tohoku Journal of Experimental Medicine, Vol. 50, Nos. 3 & 4, 1949.

Non-existence of the Direct Nervous Connection between the Vagal Nerves and the Suprarenal Glands in Dogs.

By

Siro Okuda.
(奥田 四郎)

(From the Physiological Laboratory of Prof. Y. Satake, Tohoku University, Sendai.)

(Received for publication, November 21, 1946)

The clear demonstrations of a direct branch to the left suprarenal gland from the vagus cord in the human cadaver, made by Uchida (1) and by Teitelbaum (2) have led me to try it in canine cadavers, but in vain, as given in the following paragraphs. Other description (3, 4) of this kind of observations on dogs has given us no clear impression.

It may be added in passing that it is a well-established fact that stimulation of the vagus has no effect on the epinephrine output from the suprarenal glands, and there is a degeneration experiment which checks well with it (5).

Methods.

After removal of the ventral walls of the thorax and abdomen, the upper half of the thorax and the lower half of the abdomen were out off and the portion to be examined was placed over night in 0.5% acetic acid solution, by which process the nerves were easily recognized as white glistening strands.

Dissection was undertaken in co-operation with Dr. K. Hasebe, Professor of the Anatomical Laboratory in this University at that time.

Results.

Dog No. 1 ♂.

The vagus divides into 2 stems, i.e. ventral and dorsal, which pass through the esophageal opening of the diaphragm. The ventral stem, sending the main branches through the layer of the omentum minor along the lesser curvature, reaches the ventral surface of the stomach. One of these branches is found to anastomose with a fibre from the dorsal trunk in the region of the cardia and to send a hepatic twig besides. But there is no fibre directly reaching the left suprarenal.

The dorsal stem divides further into 2 portions. The first (gastric portion) reaches the cardia and even the pylorus through the left gastro-pancreatic fold. The main branches are found running along the lesser curvature. The second portion runs into both left and right celiac plexi, which are connected with its fibres intimately, and communicates with the suprarenals by many fibres through the celiac ganglion. But there was found no direct connection between the vagal nerve and the left or right suprarenal.

Dog No. 2 ♂.

There exists no connection between the ventral trunk or its branches and the celiac plexus or the suprarenals. The two branches from the dorsal trunk run into a small ganglia and then reach the left celiac plexus. Further, these branches are connected with the left celiac ganglion and the left suprarenal respectively, but there is no direct connection between the vagus nerve and the left suprarenal. Between the left suprarenal and the left celiac ganglia, there is recognized intimate connection by fibres. There exists no direct connection between the right suprarenal and the dorsal trunk or its branches. All connection are made only through the right celiac ganglia.

Dog No. 3 ♂.

In this case, there is no nerve connection between the ventral trunk or its branches and the left or right suprarenal. The fine fibres of both trunks anastomose in the region of the cardia. A fine fibre, starting from the dorsal trunk and running straight towards the left suprarenal, reaches the left celiac ganglion and further, in the same direction, the upper portion of the left suprarenal. Since there is the left celiac ganglion between the connection of the dorsal trunk and the left suprarenal, the present writer can not recognize it as a direct connection. Besides, there are many branches connecting the dorsal trunk with the left celiac ganglion. Also between the right suprarenal and the dorsal trunk, there exists no direct connection, but there are many fibres connecting the dorsal trunk with the right celiac ganglion and the right celiac ganglion with the right suprarenal.

Conclusion.

In the dog, the existence of a direct nervous connection between the vagus and the suprarenal gland which was reported by some observers was not confirmed.

References.

1) Uchida, Acta Scholae Med. Kioto, 1927–28, **10**, 481. 2) Teitelbaum, Anat. Record, 1932–33, **55**, 297. 3) Hoshi, Mitt. üb. allg. Path. u. path. Anat., 1927, **3**, 328. Kollmann, Quoted from (5), p. 19 f. 5) McCrea, J. of Anat., 1924–25, **59**, 18.

The Tohoku Journal of Experimental Medicine, Vol. 50, Nos. 3 & 4, 1949.

Some Observations of the Gastric Secretion in Dogs on Fictitious Feeding.

By

Siro Okuda.

(奥田 四郎)

(From the Physiological Laboratory of Prof. Y. Satake, Tohoku University, Sendai.)

(Received for publication, November 21, 1946)

In attempting to know the cephalic phase of the gastric secretion in dogs when food-stuffs are given in the amounts corresponding to one third of the basal metabolism per diem rê the fuel value, we were able to obtain some new data.

In 6 dogs of 11–17 kilos, the stomach fistula or the Pavlov pouch was prepared, and some days later the esophagotomy was done. After the operations cod liver oil and milk were given twice a day besides the normal laboratory diet. After full recovery from the last operation the animals were experimented on.

About 20 hours after the previous feeding, the animals was brought into a quiet room, and tepid water was introduced into the stomach by a rubber tube through the lower esophageal fistula in order to remove the rest of food staying in the stomach, the irrigation being continued until the water coming out of the stomach fistula becomes less acid to litmus. 0.5–1 hour later the experiment was started. All the methods are given in a paper of Hirohata (I). "Sukiyaki" means beef cooked in a Japanese fashion; here 200 g of beef was boiled with soy, sugar, welsh onion, and the latter was removed after having been boiled.

I

It will be attempted at first to elucidate from the materials at hand what part of the whole gastric secretion is played by the cephalic phase.

In Table I are epitomized the gastric secretions, reckoned from the data obtained from the Dog Mukaiyama of Hirohata (on p. 110) with the Pavlov pouch, Dog No. 4 with the Pavlov pouch and esophagotomy (average of 3 experiments), and Dogs No. 3 (19. 11. 40) and No. 8 (18. VI. 40) with the stomach fistula and esophagotomy. Raw beef was given in the amount corresponding to one third of the total calories per diem. It was taken by Dog M. in I minute-, and by the others in 3 minute-fictitious feeding.

TABLE I.

Gastric secretion in the dogs, with the Pavlov fistula with or without esophagotomy or with the stomach fistula with esophagotomy, on the sham feeding with raw beef of the amount corresponding to 1/3 of the basal metabolism per diem.

	Gastric juice in cc.						
	Before	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
Dog Mukaiyama ♀, Pavlov pouch	2	15	8	6	5.5	4	2
Dog 4 ♀, Pavlov, esophagotomy	4	21	15	6	1	1	
Dog 3 ♂, stomach fistula, esophagotomy	0.5	17	14	3	1	1	0
Dog 8 ♀, 〃 〃	6	21	18	3	3	2	1

Quantities of juice in Dogs 3 & 8 here given are 1/5 of the actual, the areas of the stomach and the Pavlov pouch being taken into account roughly.

Although it is not to be denied that the materials were chosen somewhat arbitrarily, it may be justified to draw therefrom such a conclusion that the secretion covering the first two hours, the larger part of the secretion, is chiefly of cephalic origin, and it is 3 hours after the taking of food that the chemical phase of gastric secretion comes into sight for the first time. In the fourth and fifth hour the secretion from the Pavlov pouch in the dog without suffering from the esophagotomy is decidedly larger than the dogs with it. It must be assumed, as a matter of course, that the gastric juice can be secreted in such a case, as the sham feeding, also by the chemical mechanism, because the juice poured out by the cephalic mechanism may flows into the duodenum through the pyloric part, though probably small.

II

It is natural that the appetite, taste, likes and dislikes have great influence upon the amount or velocity of gastric secretion of the cephalic phase, and consequently that of the gastric secretion as the whole. Some examples will be given below.

Our dogs, except Dog No. 3, as well as all the dogs of Hirohata did not like to take plain boiled rice, which is the most common and favorite food for the Japanese as bread for the European. This peculiar taste is due to the fact that in this laboratory we give it mixed with other foodstuffs strongly seasoned. Dog No. 3, which had good appetite for all food-stuffs, had it also for plain boiled rice. On 3 minute- fictitious feeding of this animal the gastric juice was secreted in an amount of 130 cc on raw beef, 150 cc on beef-steak, 130 cc on "sukiyaki," 150 cc on fresh fish and on fermented soy-beans, and 166 cc on plain boiled rice. In passing it might be added: We can make a dog like it through training,

as to be easily expected and in fact as was experienced by Mr. K. Tamabuti in this laboratory on a later occasion.

Similar experience was made of fermented soy-beans: My Dog No. 3 and Dog Mukaiyama in Hirohata's research yielded a great amount of gastric juice, while 3 other dogs of H. secreted only a small quantity (p. 127), and the other dogs in my hand did not like this foodstuff at all.

That beef-steak always acts more powerful in producing the secretion of gastric juice, though not excesisvely, than raw beef or "sukiyaki," with the same quantity of fuel value, might be due to a better taste of the former. In Dog No. 1, when they were given until the dog discontinued to take them, 500 cc (250–750 cc in 3 experiments) were collected in 6 hours on beef-steak, 400 cc (250–500 cc) in 4 hours on raw beef, and the same (300–600 cc) in 5 hours on "sukiyaki"; in Dog No. 3 the food-stuffs were given only for 3 minutes, then 150 cc juice were secreted on beef-steak in 2.5 hrs, and 130 cc on raw beef or on "sukiyaki" in 3 hrs.

III

The fact is worthy of further discussion, that fictitious feeding extending for such a long time such as 30 minutes or 1.5–2 hours is not capable to bring about an abundant secretion of gastric juice in proportion to the length of time of feeding in contrast to the outcome of a brief fictitious feeding such as 3 minutes.

In Dog No. 1, the fictitions feeding with "sukiyaki" until the dog stopped finally to take it, a matter of 1.5–2 hrs., yielded 430 cc of gastric juice, while that of 3 minute feeding evoked a secretion of 250 cc. The secretion covered 4.5 hrs in the former, 3 cases in number, and 2.5 hrs in the latter, 1 case. In Dog No. 8, the fictitious feeding of 30 minutes with raw beef produced about 300 cc of stomach juice in 5–6 hrs, and that of 3 minutes 120 cc in 2 hrs.

In analyzing this phenomenon the following factors are to be taken into account at least: first, the effect of appetite upon the gastric secretion, that is, the potency of fresh appetite in the beginning of feeding, and secondly a long after-action or after-discharge of the central mechanism of the secretory nerve for the gastric glands.

How long this after-discharge of the central mechanism for the gastric secretion continues, in contrast with that of the salivary secretion is to be seen from a following example: In Dog No. 3, the saliva flowing out from the upper opening of the esophageal fistula was measured simultaneously with the gastric juice; "sukiyaki," salted salmon (2 times) and fermented soy-beans were given for 3 minutes. Then, the saliva flowed out soon in abundance, but stopped also soon, viz. the outflow

lasted only five minutes, never longer, while the gastric juice came out for 3–4 hrs.

In connection with this long lasting after-discharge, I had a chance to see the very long persistence of the conditional " time " reflex of gastric secretion, also in contrast to that of the salivary secretion.

In our laboratory, dogs are fed routinely once every day at 2 or 3 o'clock p.m. Dog No. 4 was fed after the esophagotomy twice, at 11 a.m. and at 2 or 3 p.m. In the interdigestive period, I came to see that Dog No. 3 secreted the gastric juice at about 3 o'clock p.m., about ten cc in 30 minutes, and Dog No. 4 also a similar quantity at the times, twice as above mentioned. Afterwards they were fed at 4 o'clock only; then a new period of secretion appeared in addition to those above mentioned. Four months later the last figure, with two or three periods of gastric secretion, was still to be observed. That is, the conditional "time" reflex of gastric secretion persists extraordinarily long.

IV

After a series of sham feeding had been done with Dog No. 3, as aobve, the Eck fistula was prepared, and the same kinds of experiments were further carried out.

Then we came to see an augmentation of the gastric secretion, but the magnitude of augmentation and its course differ from those seen by Ka (2) and others, who experimented on the dogs with the Pavlov pouch. In the latter case, the augmentation is of much larger scale, viz. 3–10 times as large as the normal control, and the augmentation appears not only in the whole period, but it is especially large in the former period of secretion, and the secretion lasts somewhat longer.

In the present sham feeding experiments the augmentation is not only small in the magnitude, but the period when it appears is limited in the latter half of secretion, or in the portions taken in the third 30-minutes to the sixth. In the fourth 30-minutes the secretion is much greater than the control, often doubles the latter. This is to be detectable easily from the data given in the upper half of Table II.

The lower half of Table II shows another series of experiments, done on Dog No. 4, in which the portal vein was made operatively narrower at a portion above the pancreatic vein, instead of having prepared the Eck fistula. After a good recovery of the animal the fictitious feeding was repeated with an exaggerated secretion, and this time the augmentation took quite a similar figure as in the experiments of Ka, for example, with the Pavlov pouch and the Eck fistula. The results are therefore different from those above given in the upper half of Table II, that is, those obtainable in the fictitions feeding before and after having established the Eck fistula.

TABLE II.

Gastric secretion by the sham feeding (3 minutes) after preparation of the Eck fistula (Dog 3) or constriction of the portal vein (Dog 4).

	Foods, No. of exp.				Gastric juice in cc (average) in 30 minute period: Before	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	Total
Dog 3: Stomach fistula, esophagotomy, Eck	Raw beef	3	Before	Eck	3	40	46	27	13	6	9	7	3	6	6	2	1	166
		2	After	Eck	25	50	38	38	30	23	19	12	6	12	6	8	10	252
	Beef steak	3	Before	Eck	6	44	37	31	18	12	9	5	9	6	7	3	5	186
		2	After	Eck	18	37	36	29	22	22	16	18	17	15	20	13	10	255
	Salted Salomon	3	Before	Eck	3	46	33	18	12	18	11	10	8	5	7	6	2	176
		2	After	Eck	12	36	33	38	20	23	15	12	13	9	12	11	10	232
	Boiled rice	3	Before	Eck	4	38	38	19	12	12	14	13	11	9	7	9	2	184
		2	After	Eck	19	53	35	28	27	23	23	17	12	10	9	9	7	253
	Fermented soy beans	3	Before	Eck	3	38	41	29	18	15	11	15	15	9	6	4	5	206
		2	After	Eck	17	52	40	38	26	25	19	18	14	9	4	6	9	260
Dog 4: Pavlov pouch, esophagotomy, constriction of v. portae	Raw beef	3	Before	Constriction	1.9	8.3	13.0	7.4	7.0	3.3	2.5	0.8	0.5	0.8	0.7	0.7	0.7	45.7
		2	After	Constriction	3.4	27.5	20.0	15	8	16.5	12	8.7	7.8	7.7	5.5	3.6	2.5	134.8
	Salted salmon	3	Before	"	1.6	7.7	8.0	4.7	3.8	4.2	4.5	4.3	3.8	3.3	1.5	1.3	1.1	48.2
		2	After	"	3.4	8.0	26.0	18.3	15.9	15.0	11.8	7.5	5.5	3.5	1.8	0.8	2.5	116.6

That the acidity, free and total, of the gastric juice increases in general as the velocity of its secretion does so, and the digestive power does not usually undergo any definite variation, was seen throughout all the present experiments. For example, the correlation between the quantity of gstric juice, secreted on receiving food-stuffs, and its acidities were almost same in the sham feedings before and after having prepared the Eck-fistula.

Summary

The present report consists of the data, yielded in the dogs with the stomach fistula or the Pavlov pouch, with the esophageal fistula. Further, in such a dog the Eck fistula was prepared, and in another the portal vein was constricted above the upper mesenterial vein. The results may be summarized as follows:

In the gastric secretion the cephalic phase makes the essential portion in the whole course, especially its former part being chiefly, at least, of the nervous origin.

The facts, that a certain food-stuffs, such as plain boiled rice, fermented soja-beans, etc, are incapable in certain dogs to cause an abundant output of gastric juice, are solely due to the fact that they evokes only poor appetite. If those dogs will be accustomed to take those food-stuffs, then gastric secretion will be increased by and by, and finally reach maximum amount just as by other stuffs, which evoke always good appetite, and accordingly an abundant outflow of gastric juice.

The fact that the fictitious feeding of a brief period, such as 3 minutes, causes a long continuned secretion of gastric juice, such as two to three hours, contrary to a brief secretion of saliva, such as 5 minutes, on the same procedure, shows a long persistence of the central mechanism of gastric secretion. In accordance with this fact the conditional reflex of gastric secretion does not disappear easily, after the conditional stimulus has been discontinued, but continues considerably long, also contrast to the salivary secretion.

The establishment of the Eck fistula incrases the gastric secretion on the fictitious feeding, but only in the latter half of the secretion period, and in a relatively small amount, contrary to the experiments with the Pavlov pouch.

On the other hand, the constriction of the portal vein above the mesenterial vein causes an abundant outflow of gastric juice, just as from the dog with the Pavlov pouch and the Eck fistula.

Some considerations or further experiments are needed to explain both data, just mentioned, and the data on the gastric secretion from the dogs with the Eck fistula, reported by Lebedinskaja, Gerez and Weiss, Murata (3), and Ka (2), altogether.

References.

1) Hirohata, Tohoku J. of Exp. Med., 1936, **28**, 106; 2) Ka, Ibid., 1939, **36**, 475, 3) Lebedinskaja, Ztschr. f. ges. exp. Med., 1933, **88**, 264; Gerez and Weiss, Ibid., 1937, **100**, 281; Murata, Zikken Shokakibyogaku, 1936, **11**, 305.

Studies on Melanin.

IV. The Influences of Hormones and Others upon Melanin Production. Preliminary Report.

By

Masahiko Watanabe.

(渡邊 正彦)

(From the Department of Dermatology, Faculty of Medicine, Tohoku University, Sendai. Director: Prof. M. Ito.)

(Received for publication, April 21, 1949)

The auther examined the influences of hormones and poisons of vegetable nervous system upon melanin production by methods similar to that of Nakajo (previously reported). The results obtained were the following.

1. Adrenalin, ephedrin, such wake-amines as 1-phenyl-2-methyl aminopropane hydrochloride can produce temporary decoloration in black gold-fish. Total and permanent bleaching of melanin, however, could not be achieved with these substances.

2. Pituitary hormone of the anterior lobe, porphyrine and acetic acid produced black pigment in yellow-red goldfish. Other substances examined (thyoid hormone, pituitary hormone of the posterior lobe, acetylcholine etc.) were found useless.

From these facts it is clear that complete decoloration of melanin is difficulty caused by injection of melanophore-diffusing substances, but a special bio-catalytic substance such as hydroquinone is required for this purpose (see previous reports of this series of investigation).

This article was abstracted from the original article in Tohoku Igaku Zasshi (Tohoku Med. J.), 1947, **37**. The details will be published in this journal later.

The Tohoku Journal of Experimental Medicine, Vol. 50, Nos. 3 & 4, 1949.

Biochemical Studies on Carbohydrates.

CII. On the Jelly of Toad and Frog Eggs.

First Report.

By

Noboru Hiyama.

(檜山　登)

(From the Medico-Chemical Institute, Tohoku Imperial University, Sendai. Director: Prof. H. Masamune.)

(Received for publication, December 1, 1946)

Having worked on frog oviducts, Schulz and Ditthorn[1] said that the jelly-forming protein is made up of galactosamine and an amino acid-grouping. Their belief that the amino sugar in this protein is galactosamine rested on a false basis that mucic acid had been obtained after oxidation of the oviduct and spawn with nitric acid. Mucic acid is obtainable from galactose but not from galactosamine. Nevertheless, the hexosamine hydrochloride, which they separated, exhibited different solubility than glucosamine hydrochloride and is not accepted as identical with the latter hexosamine, although Schulz replaced the claim later on with Becker[2] (1935) for the opinion that the above protein involves glucosamine and galactose in an equimolecular proportion. He always did not try to isolate the protein, but derived the recent conclusion from analysis of the dry powder of the oviduct which they also took as a whole for the aim of identifying the sugars.

Whether the mucinogen to be secreted for covering the eggs is the only carbohydrate-containing protein in the oviduct is extremely doubtful, because according to our experiences animal organs have in general a glycidamin or glycidamins constructing the proper tissue. In the oviduct therefore one may assume that there are present at least two glycidamins, one the mucinogen and the other the glycidamin playing an architichtonic rôle and that glucosamine of Schulz and Becker and "galactosamine" of Schulz and Ditthorn originated in different glycidamins. So when Schulz and Becker obtained glucosamine hydrochloride from the oviduct hydrolysate as crystalline deposits, "galactosamine" hydrochloride, which was much more soluble, is possible to have reamined in the mother liquid. Similar suspicion might be given as to the nitric acid-oxidation derivatives of these amino sugars of which the authors separated norisosaccharic acid but

not 2,5-anhydrotalomucic acid. In the earlier experiment, Schulz could not separate glucosamine which readily cyrstallizes, but the amount of the jelly-forming glycidamin in the gland has close relationship with rut, and in the season when it is produced abundantly, " galactosamine," if this is its constituent, will predominate over glucosamine in yield so that the former is missed.

Before the investigations referred to, Giacosa[3] and Wolfenden[4] had studied the protein from the envelopes of deposited frog egges and found it become strongly reducing to copper salts on acid hydrolysis. In addition, Wolfenden obtained non-fermenting sugar-like crystals with sweet taste but did not follow up its nature, only describing, " it "—the crystalline mass—" does not appear to be a carbohydrate, since it yields ammonia."

We have recently discovered A group-specifity of the glycidamin from the outer coverings of toad eggs, notwithstanding that the glycidamin of frog egg coverings was devoid of this potency. The finding led us to take up the research of these proteins again, particularly in connection with the carbohydrate in them, because the anti-isohemagglutinative activity of glycidamins is assumed to consist mostly in this moiety (cf. Masamune[5]).

Experimental.

Preparation of Mucin and Mucinogen from Toad- and Frog-Egg Jelly.

The glycidamin from the outer coverings of toad (Bufo vulgaris formosus Boulenger) eggs.

Eggs deposited in ponds were collected and stood in a vessel with constant change of water. On appearance from the cords of polypous processes with simultaneous movement of the eggs thereinto, the coverings were dissected and the jelly separated from the eggs as well as from their outer thin layer stained. The jelly was then strained through loose cotton gauze, wrapped in a cotton bag and washed for several days in a stream of tap water.

1. To 1 *l.* of the jelly was added 0.1 n. HCl to pH 4.0 (42 cc. of the hydrochloric acid solution were used). The jelly began to contract in 30 minutes. Centrifuged after 10 hours. The centrifugate (420 cc.) was washed twice with double the volume of water acidulated similarly, then with alcohol many times and finally with ether, and dried in vacuo—mucinogen hydrochloride. Crop, 2.3 gm.

2. A second 1 *l.* portion of the jelly was caused to shrink by standing with 100 cc. of 30% acetic acid (pH of the mixture, 2.0) for 24 hours and centrifuged. The volume was reduced to one third. It was agitated twice in two volumes of 3% acetic acid and then once in alcohol, extending each

for several hours. It was further washed with many changes of alcohol, and dried, after replacing alcohol with ether, in vacuo—mucinogen acetate. 1.2 gm. yield was obtained.

3. Mucin. A third 400 cc. portion of the jelly was suspended in an equal volume of water and the saturated $Ca(OH)_2$ added up to pH 8.5 (15 cc. of the $Ca(OH)_2$ solution were required) and stirred up. In one hour pH was lowered to 8.0. By further addition of 5 cc. of the $Ca(OH)_2$ solution and 2 hours' agitation, the jelly dissolved into a nearly homogeneous solution, which was percolated into an equal volume of 10% acetic acid. The precipitated flakes were washed with many portions of 3% acetic acid, until calcium was not detected anymore, and subsequently with alcohol and ether and dried. The room temperature throughout the course was below 20°C. Crop, 0.35 gm.

The glycidamin from the envelopes of frog (Rana japonica Guenther) eggs.

Fresh clean eggs (the coats of the eggs deposited among water grasses are not stained and hence are favourable for the purpose) were washed under running water and subjected without delay to mechanical separation of the jelly which was achieved easily by suction through a narrow glass tube.

The mucinogen hydrochloride and acetate were prepared similarly as those from the coverings of toad eggs. The average yields were 2.2 and 2.3 gm. respectively per *l.* of the jelly.

Tests and Analyses of the Glycidamins and Identification of the Hexose.

In qualitative aspects the glycidamins resembled mutually:—

Molisch test gave intensive violet colour. By the *p*-dimethylaminobenzaldehyde reagent of Ehrlich was produced a red dye from them immediately as well as after treatment with hot alkali (Osaki and Turumi[6]). Widsoe-Tollens, Oshima-Tollens and Rosenthaler tests (for methylpentose) were given (tested on the distillate with hydrochloric acid). Neuberg and Saneyoshi (for hexuronic acid) and aniline acetate and Bial's orcinol (for pentose) tests resulted in negative. Phosphorus and hydrolysable sulfur were barely detected.

Quantitative analyses gave figures embodied in Table I. The colour intensities developed by the indole, orinol and diphenylamine methods coincided fairly well with one another as expressed as galactose. The methylpentose content thus appeared minute. Galactose was separated as methylphenylhydrazone after hydrolysis and fractioning of toad mucinogen (1 gm.) in the manner described by Turumi and Sasaki[7]. When recrystallized twice from 95% alcohol, it melted constant at 190°C, and the

TABLE I.

Quantitative Analyses of Mucinogens and Mucin from Toad Egg Jelly and Mucinogen from Frog Egg Jelly.

(In=indole method. O=orcinol method. D=diphenylamine I method.)

	Preparation	Analysis	Nintrogen	Hexosamine	Galactose				Ash
					In	O	D	Average	
Toad	Mucinogin hydrochloride Prep. I	Per cent	8.63	20.2	30.75	29.5	30.08	30.08	
		Eq. per eq. wt.		1.0				1.49	
	" " Prep. II	Per cent	8.68	19.96	30.91	30.58	29.78	30.42	4.60
		Eq. per eq. wt.		1.0				1.51	
	Mucinogen acetate Prep. I	Per cent	8.32	20.92	31.45	31.25	31.04	31.28	4.40
		Eq. per eq. wt.		1.0				1.49	
	" " Prep. II	Per cent	8.38	20.75	31.21	30.92	31.04	31.05	4.31
		Eq. per eq. wt.		1.0				1.49	
	Mucin Prep. I	Per cent	8.34	20.48	31.05	30.70	30.25	30.66	3.94
		Eq. per eq. wt.		1.0				1.50	
Frog	Mucinogen hydrochloride Prep. I	Per cent	8.86	14.39	28.83	27.72	27.50	28.02	4.36
		Eq. per eq. wt.		1.0				1.94	
	Mucinogen acetate Prep. I	Per cent	8.85	14.76	28.11	27.55	27.27	27.64	4.31
		Eq. per eq. wt.		1.0				1.88	

mixed melting point test showed no depression. Measured as a 1% solution in pyridine, it did not rotate D-light at all[8] (temp., 3°C). N (micro Dumas) 9.86% (calc., 9.85%). From the mucinogen fraction precipitated by mercuric acetate at neutral reaction, the hexosamine could not be separated.

The hexosamine and galactose were present in the molecular ratio of 2:3 in the toad mucinogen and mucin and in that of 1:2 in the frog mucinogen. The carbohydrate content diverged to a certain extent between the hydrochloride and acetate of toad mucinogen, and the corresponding content in the mucin from the same source lay intermediately. But the discrepancies are not considered essential. The both salts of the frog mucinogen showed no difference from each other in the present respect.

Group Specificness.

The substances were taken up (partially dissolved and partially suspended) in physiological saline solution and faintly alkalinized according to Sinokawa[9] to submit to the assay by our usual procedure. The mucin and mucinogen from the jelly of the toad eggs inhibited isoagglutination of A erythrocytes specifically at a dilution of $1:10^5$, whereas the mucinogen from the frog-egg-coverings exhibited no anti-agglutinative activity even at only 1:100 dilution. The used blood sera both possessed agglutinin titre 160.

Summary.

1. Glycidamins were prepared from the jelly of toad (*Bufo vulgaris formosus Boulenger*) and frog (*Rana japonica Guenther*) eggs.

2. The toad mucinogen and mucin involved hexosamine and galactose in the molecular ratio of 2:3 and the frog mucinogen in the corresponding ratio of 1:2. Methylpentose was detected but was small.

3. Galactose of the toad mucinogen hydrochloride was identified as the methylphenylhydrazone.

4. The preparations from the toad jelly were found A group-active, but those from the frog jelly not.

We are indebted to the Grant Committee for Scientific Researches of the Education Department for a grant which covered the expenses of this work.

References.

1) Schulz and Ditthorn, Z. f. physiol. Chem., 1900, **29**, 373; 1901, **32**, 428.
2) Schulz and Becker, Bioch. Zeits., 1935, **280**, 217.
3) Giacosa, Z. f. physiol. Chem., 1883, **7**, 40.
4) Wolfenden, J. Physiol., 1884, **5**, 91.
5) Masamune, Ketsuekigaku Togikai Hokoku, Saishin-Igaku Sha, Osaka, 1948, I, 180.

6) Osaki and Turumi, Tohoku J. Exp. Med., 1947, **49**, 11.
7) Turumi and Sasaki, ibid., 1947, **49**, 1.
8) Cf. van der Haar, Anleitung zum Nachweis, zur Trennung und Bestimmung der Monosaccharide u. Aldehydsäuren, Gebrüder Borntraeger, Berlin, 1920, 166.
9) Sinokawa, Tohoku J. Exp. Med., this volume, p. 167.

The Tohoku Journal of Experimental Medicine, Vol. 50, Nos. 3 & 4, 1949.

Biochemical Studies on Carbohydrates.

CIII. On the Jelly of Toad and Frog Eggs.

Second Report: Hexosamine in the Toad Mucin.

By

Noboru Hiyama.

(檜 山 登)

(From the Medico-Chemical Institute, Tohoku Imperial University, Sendai. Director: Prof. H. Masamune.)

(Received for publication, December 1, 1946)

From the mucinogen hydrochloride from the coats of toad eggs[1] was isolated the amino sugar as crystalline hydrochloride and identified with chondrosamine by analysis, measurement of its melting point and optical rotatory power and also as phenylosazone and the 2-hydroxynaphthylidene derivative. The presence of this hexosamine in the non-uronic acid-containing glycidamins of chondro- and osseo-mucoid was mentioned many years ago by Hisamura[2], but this has been disproved recently by the writer[3]. On the other hand, Schulz[4] once conjectured the occurrence of galactosamine in the jelly-forming protein of the frog oviduct, but, as pointed out in the first report, the argument was not based on reasonable facts. The present experiment unobjectionably established the participation of chondrosamine in the make-up of some glycidamins which form a particular class from this stand point.

Experimental.

Separation of Chondrosamine Hydrochloride.

5 gm. of the mucinogen hydrochloride (see the first report) were taken up in 150 cc. of 30% sulfuric acid by slight warming on a water bath and then boiled on a sand bath under reflux for 14 hours. When cold, the black sediment was centrifuged off, and the dark brown supernatant liquid and the washings of the centrifugate were combined and after removing sulfuric acid with a just sufficient quantity of the saturated $Ba(OH)_2$, distilled under diminished pressure to about 10 cc. The concentrated solution was extracted with butanol in a Dakin's apparatus continuously for 20 hours, and the syrupy residue diluted to about 20 cc., decolourized with animal charcoal and subjected again to vacuum distillation ($<40°C$).

When almost dried up, the remainder was dissolved in 1–2 cc. of 20% hydrochloric acid and diluted with 5–10 cc. of absolute alcohol, and acetone added in small portions until faint turbidity occurred. On standing after scratching the vessel wall, chondrosamine hydrochloride began to separate in thin needles. Additional acetone was added from time to time until no more crystallization took place, when the deposits were separated on a suction funnel, washed with absolute alcohol and dried. Yield, 0.3–0.45 gm.

After one reprecipitation from 1.5 cc. of 20% HCl[5] with absolute alcohol, the crystals were purified further on from absolute alcohol as follows. They were taken up in a large volume of boiling absolute alcohol, transferred to a beaker and evaporated gradually on a water bath to a small volume, whereupon crystalline deposits appeared. To complete the crystallization, the beaker was placed in a desiccator (H_2SO_4) under normal pressure overnight and the deposits were collected on a Hirsch funnel and washed with cold absolute alcohol.

The product began to change brown at about 165°C and decomposed at 180°C[6] under gas evolution. No loss of water happened when it was heated at 100–110°C at a reduced pressure for 4 hours. Sulfate test negative, chloride test positive. On burning it left no ash.

N (micro Kjeldahl).

2.075 mgm. substance, 0.958 cc. 0.01 n. acid required for titration.

$C_6H_{13}NO_5 \cdot HCl$: Found, 6.46%; calc., 6.50%.

NH_2-N (micro Van Slyke).

4.15 mgm. substance, 0.467 cc. N_2(762 mm., 15°C).

$C_6H_{11}O_5NH_2 \cdot HCl$: Found, 6.57%; calc., 6.50%.

When tested in Hamasato and Akakura method, the red colouration developed by dimethylaminobenzaldehyde was as strong as the corresponding colouration with glucosamine hydrochloride.

Optical rotatory power in water.

30.9 mgm. of the substance were taken up in 3 cc. of water and the optical rotation measured immediately after dissolution and afterwards. Temperature, 3°C.

Time after dissolution	α	$[\alpha]_D^3 = \frac{\alpha \times 3.0274}{0.0309 \times 1.0042 \times 1}$
min.	degree	degree
5	+1.155	+112.7
14	+1.101	+107.4
27	+1.061	+103.5
90	+0.995	+97.8
60×3	+0.976	+95.2
60×5	+0.971	+94.7
60×10	+0.973	+94.9
60×24	+0.971	+94.7
60×27	+0.972	+94.8

The initial $[\alpha]_D$ was in the neighbourhood of Levene's value of the α-form of chondrosamine hydrochloride.

Phenylosazone of Chondrosamine from the Toad Mucinogen Hydrochloride.

0.2 gm. of the hexosamine hydrochloride was dissolved in 10 cc. of water and after addition of 0.13 gm. of crystalline sodium acetate, 1 cc. of 50% acetic acid and 0.4 gm. of phenylhydrazine, heated under reflux in boiling water for 6 hours. Crystallization (yellow crystals) began after about 3 hours from the commencement of heating and thereafter proceeded very slowly. The flask was allowed to remain in the bath after the gas had been turned out, and further stood in air overnight. The osazone was washed on a suction filter with water, with 30% alcohol and with acetone in sequence. The yield was small, amounting only to 25 mgm.

This was suspended in 5 cc. of water, boiled on an asbestos and dissolved with the smallest possible quantity of pyridine with subsequent gradual cooling. Bright yellow needles obtained were washed with dilute pyridine (similarly diluted as the mother liquid), with water and 30% alcohol and dried in a vacuum desiccator ($CaCl_2$). The amount of substance was reduced to 16 mgm.

It melted at 198°C and broke up at 200°C[7]. The mixed melting point test with the phenylosazone from galactose (m.p. 199°C; decomp. 200°C) showed no depression.

N (micro Dumas).
4.319 mgm. substance, 0.553 cc. N_2 (13.5°C, 762.85 mm.).
$C_{18}H_{22}O_4N_4$. Found, 15.35%; calc., 15.60%.

Optical rotatory power.

10 mgm. of the phenylosazone were dissolved in a mixture of 0.4 cc. of pyridine and 0.6 cc. of absolute alcohol according to Neuberg[8] and rotation of D-light measured in a 1 dm. long tube. Temperature, 6.5°C. Immediately (10 minutes) after dissolution, α was +0.74°[7]. Later determination was impossible due to browning of the solution[9]. The rotation by the phenylosazone of galactose was as follows (room temperature, 0–4°C)[7]:

	Time after dissolution (h.)				
	1/6	24	48	72	96
α (degree)	+0.76	+0.66	+0.51	+0.40	+0.40

2-Hydroxynaphthylidene Derivative of Chondrosamine from Toad Mucinogen Hydrochloride.

90 mgm. of the hexosamine hydrochloride above were condensed to a Schiff's base with 2-hydroxynaphthaldehyde by following the procedure of Jolles and Morgan[10] (the excess aldehyde was removed with chloroform). The crude product (130 mgm.) melted at 165–167°C (decomp.). After two recrystallizations from 3.5 cc. methanol, the substance (yellow thin needles, 80 mgm.) melted constant at 175–176°C under decomposition. No

depression of melting point occurred, when it was mixed with the corresponding derivative of chondrosamine from chondroitinsulfuric acid (s. below). N (micro Kjeldahl), 4.12% (calc. for $C_{17}H_{19}O_6N$, 4.20%).

The optical rotation in methanol was $[\alpha]_D^{17}=\frac{0.801\times100}{0.37\times1}=+216.5°$ at the 11th minute after dissolution and $[\alpha]_D^{17}=\frac{0.742\times100}{0.37\times1}=+200.5°$ at equilibrium (24 hours after dissolution).

From chondroitinsulfuric acid (Jorpes) chondrosamine hydrochloride was prepared by hydrolysis of the former with HCl, to which $SnCl_2$ had been added, removal of Sn with H_2S and condensation followed by separation with methanol. The product was the β-form[11] [initial specific rotation $[\alpha]_D^4=+47.9°$, equilibrium rotation $[\alpha]_D^4=+94.5°$, decomposition point 182°C] and its 2-hydroxynaphthylidene derivative[12] (yellow thin needles), with a melting point of 175° (decomp.) and involving 4.19% N, showed $[\alpha]_D^{17}=\frac{0.579\times100}{0.337\times1}=+171.8°$ (in methanol) 11 minutes after dissolution, which reached an equilibrium at $[\alpha]_D^{17}=\frac{0.676\times100}{0.337\times1}=+200.6°$ (24 hours after dissolution).

The above findings indicated that α- and β-stereoisomer of this Schiff's base show no depression of melting point when mixed.

Thanks are due to the Grant Committee for Scientific Researches of the Education Department for a grant in aid to us.

Bibliography and Notes.

1) Cf. the first report (Hiyama, Tohoku J. Exp. Med., this volume, 373).

2) Hisamura, J. Bioch., 1938, **28**, 217 and 473.

3) Hiyama, Tohoku J. Exp. Med., in press.

4) Schulz and Ditthorn, Zeits. f. physiol. Chem., 1900, **29**, 373.

5) The substance was dissolved in the hydrochloric acid solution by slight warming.

6) Levene, J. Biol. Chem., 1923, **57**, 337. According to this author the α-form of chondrosamine hydrochloride melts at 185°C and the β-form at 187°C.

7) Levene (J. Biol. Chem., 1916, **26**, 152) said that phenylosazone of chondrosamine melted at 201° (decomp. at 202°C) and that 0.05 gm. of it in 5 cc. of Neuberg's pyridine alcohol mixture had the initial rotation of +0.36° in a 0.5 dm. tube. In the experiments of Levene and La Forge (J. Biol. Chem., 1915, **20**, 429) the phenylosazone of galactose melted and decomposed at the temperatures same as above and the double concentrated solution rotated D-light as much as +0.73°~+0.80° immediately on dissolution, +0.32° after 8 hours and +0.34° after 24 hours of standing.

8) Neuberg, Ber. dtsch chem. Ges., 1899, **32**, 3384.

9) Another preparation which had been similarly prepared two years ago had the initial rotation +0.45° (10 min. after dissolution) which came down to +0.39° in 24 hours. Room temperature, 5°C.

10) Jolles and Morgan, Bioch. J., 1940, **34**, 1183.

11) Cf. Levene (6)).

12) Jolles and Morgan (10)): m. p. 175–178° (decomp.); $[\alpha]_{5461}$ initially +287° and at equilibrium +258°.

Studies on Melanin.

V. The Relationship between Pigment Production and Ferments Peroxydase and Indophenolase. Preliminary Report.

By

Masahiko Watanabe.
(渡邊 正彦)

(From the Department of Dermatology, Faculty of Medicine, Tohoku University, Sendai. Director: Prof. M. Ito.)

(Received for publication, April 21, 1949)

The relationship observed of pigment production and these two enzymes may be summarized:

1. Peroxydase reaction and oxydase reaction are strongly positive in cells possessed of melanogenic facility. In case of nevus cells, these reactions are stronger in pigment-free cells and weaker in cells with much pigment.

2. In the extracts of guniea pig skin, the pigmented area shows stronger reaction than the non-pigmented.

3. Histologically indophenolase granules coincide with melanin granules in form, size and location.

4. In strongly reacting areas peroxydase granules are often noticed in the protoplasma, too. This means that peroxydase is transferred from the nuclei into the protoplasma, or that inhibitory power of catalase in the protoplasma is suppressed by the presence of a large amount of peroxydase.

5. Endothelcells of capillaries give strongly positive reaction, and the excessively produced indophenolase or peroxydse-positive granules are disseminated in the neighbouring parts.

6. Peroxydase reaction of local blood is more strongly positive in pigmented nevi and in the pigmented area of guinea pig skin than in the non-pigmented part. This indicates that there are transmission of peroxydase between tissue cells and circulating myeloic leucocytes.

7. Peroxydase reaction of local blood is less positive in the area of vitiligo and alopecia areata.

8. From the above the participation of indophenolase and peroxydase in melanin production is surmised. Nuclei serve the storage of peroxydase.

This article was abstracted from the original article in Tohoku Igaku Zasshi (Tohoku med. J.), 1947, **37**. The details will be published in this journal later.

The Tohoku Journal of Experimental Medicine, Vol. 50, Nos. 3 & 4, 1949.

Biochemical Studies on Carbohydrates.

CIV. On the Jelly of Toad and Frog Eggs. Third Report: Hexosamine in the Frog Mucin.

By

Noboru Hiyama.
(檜山 登)

(From the Medico-Chemical Institute, Tohoku Imperial University, Sendai. Director: Prof. H. Masamune.)

(Received for publication, December 14, 1946)

The mucinogen hydrochloride of the outer covering of frog (*Rana japonica Guenther*) eggs was subjected to hydrolysis by boiling with hydrochloric acid containing stannous chloride. The hydrolysate was removed from tin and butanol-soluble fragments and condensed, and from the solution of the remainder in a mixture of dilute hydrochloric acid and methanol, crystalline hexosamine hydrochloride was deposited with acetone. After recrystallizations in a similar way till constant melting point, the product was revealed to be β-chondrosamine hydrochloride from the melting point, analytical figures and optical rotatory power.

The hot-alcohol soluble hexosamine hydrochloride, which Schulz and Ditthorn[1] separated from the frog oviduct, might have been identical with the present, although their assertion of its being "galactosamine" was based falsely on formation of mucic acid by nitric acid from co-present galactose in the oviduct and spawn which were submitted to the oxidation.

Experimental.

The mucinogen hydrochloride was prepared as described in the first report[2].

5 gm. of it were dissolved in 50 cc. of 20% hydrochloric acid, that involved 2 gm. of crystalline stannous chloride, on a water-bath and boiled under reflux for 14 hours on a sand-bath. The dark brown hydrolysate was diluted twice with water without antecedent removal of the black sediment, and H_2S gas passed. The excess H_2S was aerated off and the precipitates filtered with suction and washed with hot water exhaustively. The filtrate and washings were condensed together under diminished pressure at 40°C to syrupy consistency. Evaporation was repeated again and

again with water to eliminate hydrochloric acid as completely as possible and the final residue was transferred in a Dakin apparatus by the aid of 5 cc. of water and extracted for 20 hours with butanol. The solution was concentrated thereby to a thick syrup, which was diluted with water and after freeing from some insoluble part, distilled again at a low temperature until almost dried up. This remainder was taken up in 1 cc. of 20% hydrochloric acid, diluted with 5 cc. of methanol, and acetone added cautiously to produce a faint cloudiness. On standing after scratching, prismatic needles began to come out. Additional acetone was gradually added and when the crystallization ceased to progress (it took 2 weeks), the deposits were collected on a funnel. Yield, 0.4 gm.

The substance[3] was recrystallized from a mixture of 20% hydrochloric acid and methanol with acetone, and then from 20% HCl with absolute alcohol. Another recrystallization in a like manner as in the first time did not raise the melting point. The crystals had the same appearance as on first crystallization. At some 164°, it turned dark and broke up under gas evolution at 180°C.

Nitrogen (micro Kjeldahl):

3.04 mgm. substance, 1.392 cc. 0.01 n. acid required for titration.

$C_6H_{13}NO_5 \cdot HCl$. Found, 6.41%; calc., 6.50%.

Amino-nitrogen (micro Van Slyke):

6.08 mgm. substance, 0.698 cc. N_2 (746.4 mm., 19°C).

$C_6H_{11}O_5NH_2 \cdot HCl$. Found, 6.44%; calc., 6.50%.

Its colour development by the analytical method of Hamasato and Akakura compared with that of glucosamine hydrochloride.

It had the rotation as follows:—

5 minutes after dissolution,

$$[\alpha]_D^{17} = \frac{0.761 \times 0.0357}{0.0457 \times 1.006 \times 1} = +50.2°;$$

at equilibrium (24 hours after dissolution),

$$[\alpha]_D^{17} = \frac{1.429 \times 3.0357}{0.0457 \times 1.006 \times 1} = +94.3°[4].$$

The course of the mutarotation is illustrated in Table I.

On evaporating an alcoholic solution of the crystals above (β-chondrosamine hydrochloride), as when the hexosamine salt from toad mucinogen was purified (s. Second Report[4]), the compound came out for the most part in the α-form (thin needles). This decomposed at 179.5–180.5°C and possessed an initial rotation $[\alpha]_D^{22} = \frac{1.567 \times 100}{1.48 \times 1} = +105.9°$ (10 minutes after dissolution) and an equilibrium rotation $[\alpha]_D^{22} = \frac{1.399 \times 100}{1.48 \times 1} = +94.5°$ (4 hours after dissolution).

TABLE I.

Mutarotation of β-Chondrosamine Hydrochloride from Jelly of Frog Eggs.

(Temperature, 17°C)

Time after dissolution minute	α degree	$[\alpha]_D^{17}$ degree
5	+0.761	+50.2
10	+0.806	+53.2
17	+0.854	+56.4
26	+0.892	+58.9
36	+0.946	+62.4
60	+1.078	+71.1
60×2	+1.246	+82.2
60×3	+1.376	+90.8
60×4	+1.426	+94.1
60×8	+1.426	+94.1
60×24	+1.429	+94.3

The costs of this investigation were defrayed by a grant from the Grant Committee for Scientific Researches of the Education Department, which is gratefully acknowledged.

Bibliography and Notes.

1) Schulz and Ditthorn, Zeits. f. physiol. Chem., 1900, **29**, 373.

2) Hiyama, Tohoku J. Exp. Med., this volume, **373**.

3) It decomposed at about 168°C.

4) Regarding the equilibrium rotation of chondrosamine hydrochloride, confer the second report (Hiyama, Tohoku J. Exp. Med., this volume, 379). According to Levene (J. Biol. Chem., 1923, **57**, 337), it ($[\alpha]_D^{20}$) is +95°.

Structure of the A Specific Carbohydrate from Mucus Mucin of Pig Stomachs. Preliminary Note.

By

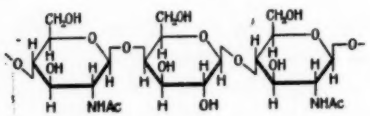
Hajime Masamune and Zensaku Yosizawa.

(正 宗 一) (吉 澤 善 作)

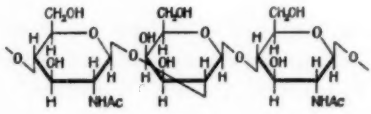
(From the Medico-Chemical Institute, Tohoku University, Sendai.)

(Received for publication, April 21, 1949)

An A-group specific carbohydrate was separated from the mucus of pig stomachs. The preparation, analyzing N 4.49%, glucosamine 44.52%, acetyl 10.77%, galactose 45.16%, ash 0.03%, was submitted to acetolysis with acetic anhydride and sulfuric acid and following fractionation by organic solvents to obtain the unit disaccharide. The product, after deacetylation (0.2 n. KOH used), began swelling at about 98°C to contract again at 135°C and upwards and melted at 175–185°C under decomposition. $[\alpha]_D^4$ in water at equilibrium was +57.81°. And it was demonstrated to be acetylglucosamine(1.5)-4-β-galactoside(1,5) by the following facts: (1) it was composed of 3.61% N, 11.21% acetyl, 47.08% galactose and 0.1% ash; (2) iodine reduction corresponded to 49.54 per cent glucose; (3) molecular weight (Rast), 386.65; (4) it gave red coloration with *p*-dimethylaminobenzaldehyde, when heated preliminarily with a dilute alkali; (5) it was digested by a snail enzyme complex involving α- and β-galactosidase, but not by α-galactosidase separated; (6) it consumed 3 mols of periodic acid per mol. The structure was further established by synthesis starting with lactose (procedures of Zemplén and of Fischer-Leuchs, N-acetylation and separation of the epimers). The product here possessed properties similar to above and the result of the mixed melting point test was perfectly identical. On the other hand, the group carbohydrate itself was found to use 11 mols of periodic acid per mol (the average molecular weight of the substance was calculated at 3,600 from hydrolysable sulfur content and iodine use). Since a part of it reaching some 10% is fulfilled by a nitrogenous non-sugar component, an octadecasaccharide, as assumed as a straight chain, is involved in it. Among possible modes of union between two adjacent structural units (the above disaccharide) in this octadecasaccharide, the ones, in which the reducing end of one of the units links C_4 or C_2 of galactose of the other, are acceptable as actual, because, in these cases, 1 mol of the octadecasaccharide reduces 11 mols of the acid and when the attachment of the mentioned nitrogenous compound and sulfuric acid are taken into consideration, 8–11 mols varying with the allocation of the rests. If C_6 or C_3 of galactose participates in the linkage, the consumption of periodic acid diverges markedly from the finding. Therefore the chain of the proper carbohydrate in the present A group substance has the structure I or II.



I



II

That β-form of glucosaminide is assigned is arbitrary. Some of the acetylglucosamine molecules take part in forming the direct-Osaki-Turumi reaction-giving nucleus.

The details will be published in forthcoming issues of this journal.

The Tohoku Journal of Experimental Medicine, Vol. 50, Nos. 3 & 4, 1949.

The Examination of Tubercle Bacilli in Sputum.

I. The Number of Tubercle Bacilli in all the Sputum that a Tuberculosis Patient Spits a Day.

By

Yasue Okada.

(岡田泰衛)

(Research Institute for Tuberculosis and Leprosy. Tohoku University, Sendai. Leaders: Prof. T. Kumagai and Prof. T. Ebina.)

(Received for publication, December 16, 1946)

Foreword.

It goes without saying that, in order to know the present condition and prognosis of a tuberculosis patient, it is most important to know the number of tubercle bacilli in the sputum of the patient in question.

Through either normal smear preparation or culture method the approximate number of the bacilli will be known.

This, however, shows only the result of the examination given to one sputum, the extremely different result may be attained when another sputum is examined. As a matter of fact, we have seen such a case very often. It is certain that a deviation thus made will lessen to a great extent, if all the sputum collected in a week and examined. But even with the sputum spat on a certain day an accurate examination may be expected.

Experimental Cases.

The experimental cases of this kind are 106 tuberclosis patients treated with

1) Artifical Pneumothorax .....54 cases
2) Phrenicoexairesis ..... 3 cases
3) Cavity suctiondrainage .....23 cases
4) Only general treatment .....26 cases

Experimental Method.

This kind of experiment was conducted by Bogen and Bennet[1], Vogt, Zappasodi and Long[2], adopting smear preparation and by Boquet[3], adopt-

ing culture method. Besides about forty years ago Hempel[4], Ellermann and Erlandsen[5] succeeded in counting the number of tubercle bacilli in the sputum.

I tried this experiment availing myself both of the smear preparation and of the culture method.

The sputum for one day is put into a graduated container, and it undergoes the following treatments.

1. *Smear Preparation Method.*

We find it difficult to find out bacilli in the sputum for one day, when the sputum in the morning ranks below Gaffky IV. In treating such sputum a kind of bacilli collecting method we adapted. After the same amount of 4% caustic soda liquid as that of the sputum is mixed with it, they are stirred to be made even. About 15 minutes are enough to do it, 2 cc or 4 cc of the mixture (which proves to contain 1 cc or 2 cc sputum) is taken into a graduated centrifugal tube and 20 or 30 cc sterilized water is put into it. For a quater of an hour works the centrifuger, making 4,000 revolutions a minute. The sediment thus made was always limited to 0.5 cc. When more than 0.2 cc sediment settles, some of the remaining upper part liquid is added to it to make the whole amount of the sediment 0.5 cc.

The rest of the remaining upper part liquid is completely mixed and 0.05 cc is taken out of the middle stratum of it and cultivated on the solid culture medium. When the sediment settles short of 0.2 cc, especially in the case where the bacilli in question are always found out only through culture method, the sediment is exclusively used. After the sediment is stirred enough and evenly, 0.05 or 0.1 cc of it is smeared 10 square centimeters wide on an object glass, dried and examined with a microscope.

As for the sputum which ranks above Gaffky V, it is gradually heated in the 37°C hot water bath, stirred, made into the same quality as carefully as possible. Then 0.05 cc or 0.1 cc of it is smeared 10 cm^2 as in the preceding examination.

Each preparation of both kinds was revealed in the 300 visual fields. The microscope used was "Olympus," ocularlens 8×, object lens 100× (1/12). When this type of microscope is used, the real area of one visual field, which is the main standard of counting the bacilli, proves to cover about 0.017 mm^2. Accordingly 10 cm^2 contain 60,000 visual fields, which leads to this: The number of the bacilli found in 300 visual fields, multiplied by 200 will be that of the bacilli in all visual fields. In this way the number of the bacilli in the whole amount of the sputum used, and accordingly both in the sputum for one day and in 1 cc sputum can be easily known.

2. *Culture method.*

The sputum made even as afore-said is diluted with 2% caustic soda liquid by degree of 10, 100, 1,000, 10,000 and 1,000,000 times. When the sputum ranks above Gaffky V, before the dilution it is mixed with the same amount of 4% caustic soda liquid in order to be sterilized. 0.05 cc of it is dropped into a piece of Oka-Katakura's Culture medium to make a culture.

The sputum which contains a smallest number of the bacilli is centrifugalized to settle the sediment in the same way as is adapted in making a smear preparation. 0.05 cc both of the sediment and of the remaining upper part liquid are taken and each of them is placed on a piece of culture medium. When a month or so has passed, its colonies are counted. Then the number of the bacilli in 1 cc and at the same time that in the sputum for one day is to be summed up.

The Result.

From various points of view it is considered that the number of the bacilli got through the above-mentioned methods may be smaller than it really is. What is especially important to be noticed is the fact that by the culture method only the living bacilli are counted.

1. *Gaffky's Table and the number of the Bacilli.*

The number of the bacilli in a smear preparation indicates that of the bacilli in a certain amount of sputum. Gaffky's Table is one of tle standards of this kinds of indication. Comparing the number of the bacilli in 1 cc sputum with that number which is shown in Gaffky's Table, it is quite natural that we should see the increase of the number of the bacilli in accordance with the number shown in Gaffky's Table.

In case of Gaffky I. 1,000 or 3,000 or even 5,000 bacilli exist in 1 cc sputum. Speaking of all visual fields, the preparation has 60,000 fields (on 10 cm^2), but in reality, I regard 300 visual fields as all fields.

In case of Gaffky II, there exists one bacillus in many visual fields, the meaning of which is pretty vague, but in this case 5,000–300,000 or so bacilli exist in 1 cc. In case of

Gaffky III exist 300,000– 500,000,
Gaffky IV ,, 500,000–1,000,000,
Gaffky V ,, 1,000,000–2,000,000,
and Gaffky VI ,, 2,000,000–3,300,000 in 1 cc.

As for VII and above it, it is considered that various kinds of difference may arise in accordance with the views of examiners, but, agreeing the greater part of Bogen's opinion, I have deciedd as follows:

TABLE I.

Gaffkys number	Number of bacilli in 1 cc sputum	Number of example	Number of bacilli in 1 days sputum	Number of example	Total
I	1.200	1	8.740	1	4
	1.335	1	9.600	1	
	2.000	1	20.000	1	
	2.900	1	53.400	1	
II	5.000–6.000	2	33.750	1	29
	8.675	1	56.800	1	
	10.000–20.000	3	200.000–500.000	6	
	30.000–40.000	3	500.000–1.000.000	3	
	60.000–70.000	2	1.000.000–2.000.000	5	
	70.000–100.000	5	2.000.000–3.000.000	3	
	100.000–150.000	6	3.000.000–4.000.000	3	
	150.000–200.000	3	5.000.000–6.000.000	3	
	200.000–300.000	4	6.127.200	1	
			12.000.000–17.000.000	3	
III	300.000–350.000	3	4.590.000	1	4
	456.000	1	15.000.000–30.000.000	3	
IV	600.000–700.000	2	1.894.896	1	7
	700.000–900.000	3	8.807.200	1	
	900.000–1.000.000	2	18.969.405	1	
			20.000.000–50.000.000	4	
V	1.000.000–1.500.000	11	15.000.000–20.000.000	2	12
	1.566.130	1	20.000.000–40.000.000	4	
			40.000.000–50.000.000	2	
			50.000.000–65.000.000	4	
VI	2.000.000–3.000.000	3	15.110.000	1	4
	2.027.000	1	35.000.000–50.000.000	3	
VII	3.425.740	1	44.640.000	1	11
	3.618.000	1	82.174.000	1	
	3.671.200	1	84.437.600	1	
	4.000.000–5.000.000	5	89.079.240	1	
	5.000.000–6.000.000	2	180.000.000	1	
	6.090.000	1	180.000.000–200.000.000	5	
			201.720.225	1	
VIII	7.214.000	1	124.280.000	1	2
	15.002.000	1	300.040.000	1	
IX	35.380.300	1	273.000.000	1	2
	27.300.000	1	431.465.400	1	
X	No example	0	No example	0	0

Gaffky VII—15 or there abouts in one visual field and in 1 cc Sputum 3,000,000–6,000,000 are counted.

Gaffky VIII—30 or thereabouts in one visual field in 1 cc. 7,000,000–15,000,000 are revealed.

Gaffky IX—100 or thereabouts in one visual field 300,000,000 or so in 1 cc.

Gaffky X—Supposing above 200 in one visual field, there may exist over 60,000,000 in 1 cc.

How many tubercle bacilli in 1 cc sputum will usually enable us to find the bacilli in a smear preparation? A number of researches have been prosecuted in this subject, but there is a considerable difference among the numbers of the bacilli indicated by the researchers.

Now I have obtained the above-mentioned result. According to Bogen, who counted the bacilli in the same way as I did, when a case is regarded as "microscopic negative," less than 1,000 bacilli are contained in 1 cc sputum. And 1,000–1,000,000 bacilli in the sputum for one day prove "negative" on some occasions and "positive" on others.

However, each of these numbers estimated is based on the number of the bacilli found through microscopic examination of the tuberculous sputum which may be quite different from the result of microscopic examination of the sputum mixed with the bacilli.

Matson[6] reports 550,000; both Corper[7] and Bilbao[8] 100,000; Schmidt-Lange[9] above 2,000,000.

All of these are the results of the microscopic examinations of the sputum mixed with the bacilli. I also tried to see how many bacilli in a smear preparation will enable the conductors of the experiments to find the bacilli accurately after all.

The first procedure is to mix the "culture negative" sputum with the tubercle bacilli emulsion in various degrees. Then next procedure to smear 0.1 cc of the mixture 10 cm^2 wide on the object glass to be dyed.

The microscope used was likewise "Olympus." On this occasion 10 cm^2 has about 100,000 visual fields, that is, 1 cc all smeared up, makes 1,000,000 visual fields.

And in the same way as before 300 visual fields of each preparation are examined. The result is shown in Table II.

This result shows as the following facts. In case of 1,000 bacilli in 1 cc, each of the four preparations prove "negative." I found some preparations "positive" in case of 2,000 and of 3,000, which was probably an accidental success. When the number of the bacilli comes up to 7,000, they can hardly be seen. Any difference is scarecely seen in this experiment while their number is between 7,000–50,000. When the number comes up to 50,000, the experiment nearly ends in success. Even in case of 100,000 some negative preparations may still be found, but it may well be said that positive ones can be found without fail when the sputum contains 100,000 bacilli in 1 cc. Within the limit of 90,000 they may rank Gaffky I. It is not until the number of the bacilli comes up to 100,000 that we find they may rank nearly Gaffky II. The number of the bacilli increases from that on, but up to 1,000,000 they rank II as yet.

Table II.

Number of bacilli in 1cc sputum	Examp.	Visual fields in which bacilli were found	Number of found bacilli	Gaff. numb.	Number of bacilli counted through microscop. examin.
1.000	1	0	0		0
	2	0	0		0
	3	0	0		0
	4	0	0		0
2.000	1	0	0		0
	2	0	0		0
	3	0	0		0
	4	1	2	I	6.666
3.000	1	0	0		0
	2	0	0		0
	3	1	1	I	3.333
	4	1	2	I	6.666
4.000	1	0	0		0
	2	0	0		0
	3	0	0		0
	4	0	0		0
5.000	1	0	0		0
	2	0	0		0
	3	0	0		0
	4	0	0		0
6.000	1	0	0		0
	2	0	0		0
	3	0	0		0
	4	0	0		0
7.000	1	0	0		0
	2	0	0		0
	3	1	1	I	3.333
	4	1	2	I	6.666
8.000	1	0	0		0
	2	0	0		0
	3	1	1	I	3.333
	4	1	2	I	6.666
9.000	1	0	0		0
	2	0	0		0
	3	1	1	I	3.333
	4	1	2	I	6.666
10.000	1	0	0		0
	2	0	0		0
	3	1	1	I	3.333
	4	2	2	I	6.666
20.000	1	0	0		0
	2	0	0		0
	3	1	1	I	3.333
	4	2	3	I	9.999
30.000	1	0	0		0
	2	0	0		0
	3	1	1	I	3.333
	4	1	1	I	3.333
40.000	1	0	0		0
	2	0	0		0
	3	0	0		0
	4	1	1	I	3.333
50.000	1	0	0		0
	2	1	1	I	3.333
	3	1	1	I	3.333
	4	2	2	I	6.666
60.000	1	0	0		0
	2	0	0		0
	3	2	2	I	6.666
	4	2	2	I	6.666
70.000	1	0	0		0
	2	1	1	I	3.333
	3	1	1	I	3.333
	4	1	1	I	6.666
80.000	1	0	0		0
	2	1	2	I	6.666
	3	1	2	I	6.666
	4	2	2	I	6.666
90.000	1	1	1	I	3.333
	2	1	1	I	3.333
	3	2	2	I	6.666
	4	3	3	I	9.999
100.000	1	0	0		0
	2	3	4	I	13.332
	3	6	6	II	19.998
	4	7	9	III	29.997
200.000	1	3	3	I	10.009
	2	4	5	II	16.665
	3	8	9	II	29.997
	4	8	10	II	33.333
300.000	1	4	4	I	13.332
	2	7	7	II	23.331
	3	12	14	II	46.662
	4	15	19	II	63.327
400.000	1	6	10	II	33.333
	2	18	25	II	83.325
	3	21	28	II	93.324
	4	23	31	II	103.323
500.000	1	36	43	II	143.319
	2	55	61	II	203.313
	3	79	88	II	293.304
	4	93	101	II	336.633
1.000.000	1	197	256	II	853.248
	2	244	282	II	939.906
2.000.000	1	294	827	IV	2.746.392
	2	300	533	III	1.776.489
3.000.000	1	300	656	IV	2.186.448
	2	300	16.360	VI	5.448.546
4.000.000	1	289	748	IV	2.493.084
	2	300	13.500	V	4.495.500
5.000.000	1	297	39.322	VII	13.194.226
	2	300	24.191	VI	8.055.603

Very many bacilli are overlooked in cases where some 500,000 are contained. On the contrary, when 2,000,000 or more are contained, not a few cases are noticed where the number of the bacilli found through microscopic examination is greater than the real number.

Juding from the result as is mentioned above, the number 550,000, which Matson point out at microscopic visible limit, is considered a little too great, but generally speaking, the difference either of the type of microscope or of the way of smearing or of the visual field will enough engender such diviations as among the above reports.

Comparing the result shown in the second table with that in the first table, nearly complete correspondence is seen both in the middle part of Gaffky II and unwards.

2. *The number of bacilli in the sputum for one day.*

The worse gets a patient, the more becomes the quantity of the sputum and accordingly the greater the number of the bacilli grows in it. This is quite natural, but we often experience that on some occasions an unexpectedly large number of bacilli are found in a small quantity of sputum and that on other occasions the bacilli cannot easily be detected in spite of a large quantity of sputum spat.

Which contains more bacilli cannot be decided through an ordinary microscopic examination. In order to know the number of the bacilli spat for one day it is quite necessary to measure the amount of the sputum to be examined.

To lessen a deviation which is likely to arise, the whole amount of sputum for one day must be examined.

However, the result of the frequent microscopic examinations runs parallel with the number of the bacilli found in the sputum for one day. As a rule the relation between Gaffky's Table and the number of the bacilli in the sputum for one day will be explained as follows:

Gaffky I —10,000–50,000 or there abouts
Gaffky II —(for the most part)
20,000–6,000,000
(some rare case)
17,000,000 (the largest number to be found here)
Gaffky III —15,000,000–30,000,000
Gaffky IV–VI—50,000,000 or there abouts
Gaffky VII —100,000,000–200,000,000 or so.
Gaffky above VIII—more than 200,000,000.

The highest record made in my experiments was 431,465,400, viz. nearly 5 mg. Boquet[3] and his supporters counted the bacilli in a culture and declared that there exist 10–125 mg bacilli in the sputum that some

patients spat 50 cc for one day. This number reported, howevre, is too enormous to be believed.

3. *The comparison between smear preparation and culture.*

Supposing the number of the bacilli found through smear preparation method is 1, we shall decide the rated number of the bacilli in the culture. When the results thus attained are compared with Gaffky's Table, it will form Table III.

Looking at the table, you will find that in Gaffky I and II most cases prove the number of the bacilli in the culture is larger than that in the

TABLE III.

Gaffky's number	Ratio of number counted through culture to that by smear method	Number of examples
I	2.5	1
	2.6	1
	3.0	1
	6.0	1
II	0.5	2
	0.8.....1.0	6
	1.1.....1.3	10
	1.7.....1.9	4
	2.0.....3.0	4
	3.0.....4.0	3
III	0.5	1
	0.8	1
	1.0	2
IV	0.3	1
	0.6	2
	1.1	3
	1.2	1
V	0.3	1
	0.7.....1.0	8
	1.1.....1.5	3
VI	0.3	1
	0.4	1
	0.5	2
VII	0.4	1
	0.8	4
	0.9	1
	1.0	5
VIII	0.3	1
	0.7	1
IX	0.1	1
	0.8	1
X	No example	0

smear preparation. Above Gaffky V this relation is always reverse.

Both of those numbers nearly equal in IV and V.

Why will such difference arise?

The following 2 answers of all are most important.

In the first place, in case of smear preparation method, as is mentioned already, the fewer are the bacilli the oftener are they likely to be overlooked.

In the second place, when a large number of bacilli are contained in the sputum, more bacilli can be countered with the smear method. Then the culture method, because only living bacilli can be cultured, while not only living but also dead bacilli can be dyed.

Summary.

Making use of the smear preparation and of culture, I tried to count up the tubercle bacilli in the sputum for one day.

The experiment ended in such result as is shown below.

The result through the smear preparation method:—

Gaffky I—10,000–50,000 bacilli in the sputum for one day
Gaffky II—20,000–6,000,000
Gaffky III—15,000,000–30,000,000
Gaffky IV–VI—50,000,000 or there abouts
Gaffky VII—100,000,000–200,000,000
Gaffky above VIII—more than 200,000,000.

Not a few cases show that the number of the bacilli in the sputum for one day differs greatly in accordance with the quantity of the sputu, though the same number of Gaffky's Table is assigned to them.

At the same time I tried to solve the problem how many bacilli in 1 cc will make a positive smear preparation.

The number of the bacilli obtained through microscopic observation of the sputum of patient runs as follows.

Gaffky I—1,000–3,000 (or 5,000)
Gaffky II—5,000–300,000
Gaffky III—300,000–500,000.

Meanwhile some 7,000 in 1 cc will hardly have the bacilli revealed under the microscope in the mixture of the sputum and the bacilli. Up to 50,000 there can be found hardly any difference, though in case of 50,000 a success is nearly always assured.

Gaffky I includes them, up to 90,000. In case of 100,000 it is quite sure to find them, which will rank Gaffky II.

The results of oth methods begin to correspond with each other at the middle part of Gaffky II.

Comparing the number of the bacilli found in a smear preparation with that in the culture, we notice that, in case of Gaffky I and II, the

former is greater than the latter and viceversa above Gaffky VI. Both of them are nearly equal in case of Gaffky IV and V.

Literature.

1) Bogen and Bennet: Amer. Rev. Tbc. 1939, **39**, 89.
2) Vogt, Zappasodi and Long. Amer. Rev. Tbc. 1940, **41**, 481.
3) Boquet and Lenci: Annales de l'Inst. Pasteur. 1940, **64**, 293.
4) Hempel: Dissert. Leipzig. 1902.
5) Ellermann u. Erlandsen: Ztschr. f. Hyg. u. Infekt. 1908, **61**, 219.
6) Matson: Beitr. z. Kl. Tbk. 1912, **24**, 193.
7) Corper: J. Amer. Med. Ass. 1928, **91**, 371.
8) Bilbao & Rafael: Zbl. f. Ges. Tbc. Forsch. 1941, **53**, 486.
9) Schmidt-Lange u. Banning, Kl. Wschr., 1941, **14**, 344.

17158 L